

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/20172> holds various files of this Leiden University dissertation.

**Author:** Hogendorf, Wouter Frederik Johan

**Title:** Synthetic methods to glycerol teichoic acids

**Issue Date:** 2012-11-22

# **Synthetic Methods to Glycerol Teichoic Acids**

PROEFSCHRIFT

ter verkrijging van  
de graad van Doctor aan de Universiteit Leiden,  
op gezag van Rector Magnificus prof. mr. P.F. van der Heijden  
volgens besluit van het College voor Promoties  
te verdedigen op donderdag 22 november 2012  
klokke 13:45 uur

door

**Wouter Frederik Johan Hogendorf**

Geboren te Leiden in 1983

# Promotiecommissie

Promotor: Prof. Dr. G.A. van der Marel

Co-promotores: Dr. J.D.C. Codée  
Dr. D.V. Filippov

Overige Leden: Prof. Dr. H.S. Overkleeft  
Prof. Dr. J. Brouwer  
Prof. Dr. J. Lugtenburg  
Prof. Dr. C.A.A. van Boeckel  
Prof. Dr. J. Huebner

*"If you want a happy ending, that depends,  
of course, on where you stop your story."*

Orson Welles

# Table of Contents

	List of Abbreviations	5
<b>Chapter 1</b>	General Introduction	7
<b>Chapter 2</b>	Synthesis of an $\alpha$ -Kojibiosyl GTA Hexamer	35
<b>Chapter 3</b>	Automated Solid Phase TA Synthesis	55
<b>Chapter 4</b>	Fluorous Linker Assisted TA Synthesis	71
<b>Chapter 5</b>	Light Fluorous Synthesis of Glucosylated GTAs	99
<b>Chapter 6</b>	Synthesis of TA-Protein Conjugates and Their Immunological Evaluation	119
<b>Chapter 7</b>	Summary and Future Prospects	131
	Nederlandse Samenvatting	145
	List of Publications	147
	Curriculum Vitae	149

# List of Abbreviations

5-BTT	5-benzylthio-1 <i>H</i> -tetrazole	ELISA	enzyme-linked immunosorbent assay
Å	Angstrom	ESI	electro spray ionization
Ac	acetyl	Et	ethyl
Ala	alanyl	eq	molar equivalents
All	allyl	Fmoc	Fluorenylmethyloxycarbonyl
ATP	adenosine triphosphate	F-Msc	[1 <i>H</i> ,1 <i>H</i> ,2 <i>H</i> ,2 <i>H</i> ]-perfluorodecylsulfonyl Ethoxycarbonyl
BOM	benzyloxymethyl	F-Psc	perfluorooctylpropylsulfonylethoxy carbonyl
BOP	benzotriazol-1-yl-oxy-tris- (dimethylamino)phosphonium hexafluorophosphate	F-Pse	perfluorooctylpropylsulfonyl ethyl
bs	broad singlet	F-SPE	fluorous solid phase extraction
BSA	bovine serum albumin	Glc	glucose
Bt	benzotriazole	GlcNAc	<i>N</i> -acetyl glucosamine
Bz	benzoyl	GTA	glycerol teichoic acid
CAN	Ceric ammonium nitrate	h	hour(s)
Cbz	carboxybenzyl	HEK	human embryonic kidney
CDP	cytidine diphosphate	HiB	<i>Haemophilus influenza</i> type b
CE	2-cyanoethyl	HIC	hydrophobic interaction chromatography
CFU	colony forming unit	HOBT	hydroxybenzotriazole
COD	1,5-cyclooctadiene	HP	high performance
COSY	correlation spectroscopy	HPLC	high performance liquid chromatography
CP	capsular polysaccharide	HRMS	high resolution mass spectrometry
CPG	controlled pore glass	HSQC	heteronuclear single quantum coherence
δ	chemical shift	Hz	hertz
d	doublet	i.v.	intravenously
DCA	dichloroacetic acid	Ig	immunoglobulin
dd	doublet of doublets	IL	interleukin
ddd	doublet of doublet of doublets	Im	imidazole
DDQ	2,3-dichloro-4,5-dicyano-1,4- benzoquinone	<i>i</i> Pr	isopropyl
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene	IR	infrared
DC	dual color	<i>J</i>	coupling constant
DCC	<i>N,N'</i> -dicyclohexylcarbodiimide	kDa	kilodalton
DCM	dichloromethane	KLH	keyhole limpet hemocyanin
DCE	1,2-dichloroethane	LCMS	liquid chromatography – mass spectrometry
DCI	4,5-dicyanoimidazole	LPS	lipopolysaccharide
DIC	<i>N,N'</i> -diisopropylcarbodiimide	LTA	lipoteichoic acid
DiPEA	<i>N,N</i> -diisopropylethylamine	m	multiplet
DMAP	4-dimethylaminopyridine	<i>m</i> -CPBA	<i>meta</i> -chloroperbenzoic acid
DMF	dimethylformamide	Me	methyl
DMT	4,4'-dimethoxytrityl	min	minutes
DNA	deoxyribonucleic acid	MMT	4-methoxytrityl
DTd	diphtheria toxoid	MS	molecular sieves
DTT	dithiothreitol		
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide		

## List of Abbreviations

---

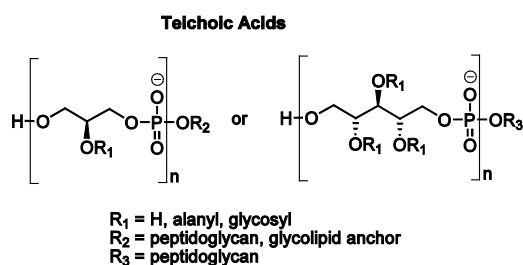
<i>m/z</i>	mass-to-charge ratio	SDS	sodium dodecyl sulfate
NMI	<i>N</i> -methylimidazole	sn	stereospecific numbering
NMR	nuclear magnetic resonance	t	triplet
NRS	normal rabbit serum	TA	teichoic acid
OPA	opsonophagocytic assay	<i>t</i> Bu	<i>tert</i> -butyl
OPIA	opsonophagocytic inhibition assay	TBAF	tetrabutylammonium fluoride
PAB	<i>para</i> -azidobenzyl	TBAI	tetrabutylammonium iodide
PAGE	polyacrylamine gel electrophoresis	TBDPS	<i>tert</i> -butyl-diphenylsilyl
PBS	phosphate buffer saline	TCA	trichloroacetic acid
PE	petroleum ether	TES	triethylsilane
PEG	polyethylene glycol	TFA	trifluoroacetic acid
Ph	phenyl	TfOH	trifluoromethanesulfonic acid
Phth	phthaloyl	Tf <sub>2</sub> O	trifluoromethanesulfonic anhydride
Piv	pivaloyl	THF	tetrahydrofuran
PMB	<i>para</i> -methoxy benzyl	TLC	thin liquid chromatography
PPTS	pyridinium <i>para</i> -toluene sulfonate	TLR	Toll-like receptor
pTsOH	<i>para</i> -toluenesulfonic acid	TMS	trimethylsilyl
pyBOP	benzotriazol-1-yl- oxytripyrrolidinophosphonium hexafluorophosphate	TNF- $\alpha$	tumor necrosis factor alpha
		TPSNT	triisopropylphenylsulfonyl nitrotriazolate
pyr	pyridine	Ts	<i>para</i> -toluenesulfonyl
q	quartet	TTBP	2,4,6-tris( <i>tert</i> -butyl)pyrimidine
RNA	ribonucleic acid	TTd	tetanus toxoid
RP	reversed phase	UDP	uridine diphosphate
RT	room temperature	UV	ultraviolet
r.t.	room temperature	WBC	white blood cell
s	singlet	WTA	wall teichoic acid
s.c.	subcutaneously		

# Chapter 1

## General introduction

### Teichoic acids: An introduction

Teichoic acids (TAs, see Figure 1), a prominent class of phosphodiester containing compounds of bacterial origin are the topic of this thesis. TAs, the name of whom is derived from the Greek “Teichos”, or city wall, are important components of the cell wall of Gram-positive bacteria. TAs comprise 30-70% of the walls of these bacteria and consist mostly of alditol (glycerol, ribitol) phosphates, non-stoichiometrically functionalized with D-alanyl and carbohydrate moieties.<sup>1-6</sup> TAs are involved in several functions necessary for survival of the bacterial species. In addition, TAs are known to interact with the host immune system, although the molecular mode of action of this process has yet to be established. Structure activity relationship studies could provide more insight in the immunological behavior of TAs but remain a difficult undertaking



**Figure 1.** Chemical structure of TAs.

as TAs isolated from biological sources generally consist of a microheterogenous mixture of compounds varying in length, glycosylation/alanylation pattern, fatty esters, linkage units etc. Well-defined fragments which can be obtained by means of chemical synthesis can be a valuable tool in the elucidation of the immunological mode of action of TAs.

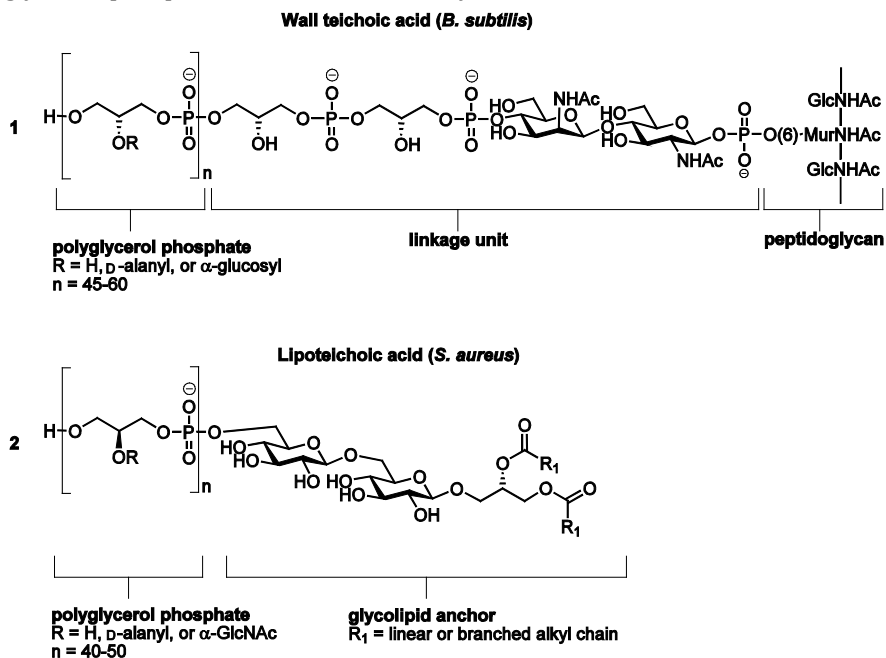
This introductory chapter presents the discovery, the structural classification, as well as physiological and immunological aspects of TAs. Additionally, it presents a selection of previously reported syntheses of TAs and an outline of the thesis.



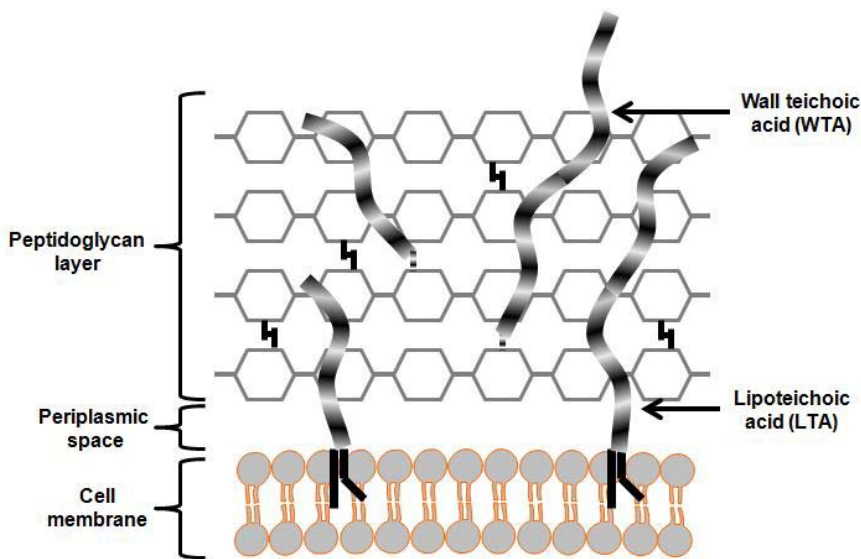
## Discovery of TA and its subclasses

The discovery of teichoic acid (TA) can be viewed as a consequence of combined evidence that was collected by research performed in the 1950s. In 1951, Leloir and coworkers identified uridine diphosphate (UDP) sugars as being the active ingredients in the biosynthesis of oligosaccharides.<sup>7</sup> With the finding of cytidine diphosphate glycerol (CDP-glycerol) and CDP-ribitol in the Gram-positive cell wall, the existence of a TA type of polymer was hypothesized.<sup>8</sup> Moreover, the total amount of phosphate present in the Gram-positive cell wall was much higher than could be attributed to the then known phosphate-containing biomolecules (nucleic acids, phospholipids etc.).<sup>9</sup> Finally, a polymer containing both ribitol phosphate and glycerol phosphate was isolated by Baddiley and coworkers from the cell wall of *Lactobacillus arabinosus*.<sup>1</sup> Further studies showed that the cell walls of Gram-positive species *Bacillus subtilis*, *Staphylococcus aureus* and *L. arabinosus* contained 30-70% of these polymers, which were named teichoic acids. In contrast, it was observed that in the Gram-negative *Escherichia coli* no TA was present at all.<sup>10-13</sup>

It was found that some TAs are connected to the peptidoglycan layer via a covalent linker that consists of a *N*-acetyl glucosamine moiety, often in combination with one or two other amino sugar residues.<sup>10</sup> This subclass of TAs is now known as wall teichoic acid (WTA) and an example of such a structure is given in Figure 2 (structure 1). Ensuing studies on *Lactobacillus casei* showed the presence of a substantial amount of polyglycerol phosphates that shared many structural similarities with the WTA,



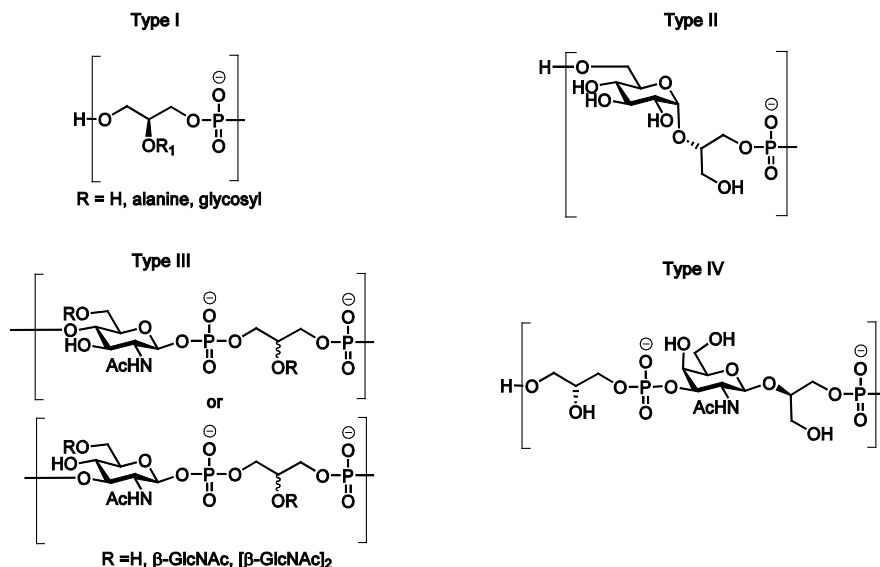
**Figure 2.** Examples of 1: WTA (*Bacillus subtilis*)<sup>4</sup> and 2: LTA (*Staphylococcus aureus*).<sup>14</sup>



**Figure 3.** Schematic representation of LTA/WTa placed in the Gram-positive cell wall.

bearing D-alanyl and various glycosyl substituents. On the basis of their discriminative lipophilicity these polymers were initially named intracellular teichoic acids.<sup>15</sup> Later it became clear that the polyglycerol phosphate chain of these TAs is terminated with a glycolipid moiety (glycosyl diacylglycerol) that, presumably, intercalates in the lipid bilayer.<sup>16</sup> Therefore their name was changed into lipoteichoic acid (LTA), of which compound **2** is an example (Figure 2). A representation of WTA and LTA in the Gram-positive cell-envelope is given in Figure 3.

Over the years, various TA structures have been identified and they have been classified by Fischer into four distinctive types, differing in the nature of the repeating units.<sup>2,17</sup> Examples of type I-IV TA structures are portrayed in Figure 4. Type I TA is ubiquitous among Gram-positive species and consists of alditol phosphate repeating units; generally poly 1,3-glycerol phosphate or poly 1,5-ribitol phosphate (see also Figure 1). The general structure of type II TA consists of poly glycosylalditol phosphate. An example is the major TA from *Bacillus stearothermophilus*, consisting of poly  $\alpha$ -glucosylglycerol phosphate, in which the glucosyl C-6 and the glycerol C-1 are linked via a phosphodiester.<sup>18</sup> Type III TA is built up from alternating repeating unit consisting of alditol phosphate and glycosyl phosphate, exemplified by the TA from *Staphylococcus hyicus* NCTC 10350.<sup>19</sup> Finally, type IV TA is characterized by alternating alditol phosphate and glycosylalditol phosphate moieties, such as the TA from several *Nocardiopsis* species.<sup>20,21</sup>



**Figure 4.** Examples of Type I-IV TA. Type I: ubiquitous in most Gram-positive species. Type II: glucosylglycerol phosphate as present in *Bacillus stearothermophilus* B65.<sup>18</sup> Type III TA from *Staphylococcus hyicus* NCTC 10350.<sup>19</sup> Type IV TA from several *Nocardiopsis* strains.<sup>20,21</sup>

## TAs in bacterial physiology

TAs are situated in the Gram-positive cell-envelope, protruding through the peptidoglycan layer and are essential components for the well-being of the bacteria as explained in the coming section of this introduction.<sup>2-6</sup> Studies on LTA-deficient *Bacillus subtilis* and *Staphylococcus aureus* mutants showed that these bacteria were only able to proliferate under a narrow range of conditions. The LTA-deficient bacteria became more susceptible to temperature stress and showed significant impairment of growth.<sup>22,23</sup> A mutant of *B. subtilis* that was prevented from WTA expression showed similar behavior. Additionally, these WTA deficient mutants were affected by normal buffer components such as citrate and had the tendency to aggregate.<sup>24-27</sup> Because species, deficient in both WTA and LTA, showed no viability it was suggested that WTA and LTA have overlapping functions and they can compensate for each other to some extent.

TAs have the capability to bind cations, especially bivalent ones (e.g.  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$ ). Upon metal ion binding, the conformation of the TAs changes, leading to an alteration of the structure and shape of the peptidoglycan/TA matrix, thereby affecting the rigidity and porosity of the cell envelope.<sup>28</sup> TAs play a vital role in the bacteria's cation homeostasis. This is illustrated by the finding that *Bacillus subtilis* boosts up its TA production in a low  $\text{Mg}^{2+}$  environment. Additionally, when the bacteria are grown under phosphate deficient conditions a significant amount of teichuronic acids, a non-phosphorylated polymer built up from negatively charged sugar uronic acids, is

produced.<sup>29</sup> It is suggested that TAs control the activity of autolysins, an important class of cell-envelope based enzymes, which are responsible for the local break down of the peptidoglycan layer to allow the bacteria to grow or divide. It is suggested that autolysins are activated by bivalent cations present in the TA.<sup>27,30</sup>

Because of their polyanionic character TAs also bind to cationic antimicrobial peptides and glycopeptides based antibiotics, thereby playing an important role in bacterial defense. TAs are also considered as targets for bacteriophages.<sup>31-33</sup>

Most TAs are randomly substituted with D-alanine esters and glycosyl moieties and the exact substitution pattern differs per Gram-positive species and can change depending on the environment. Whereas the function of glycosyl moieties on the TA chain is unknown, the role of the alanine substitution is more clear.<sup>5,6</sup> The rate of D-alanylation strongly affects the ability of TA to bind metal ions. In this respect, the group of Baddiley found that non-alanylated TA binds up to 60% more  $Mg^{2+}$  when compared to the corresponding alanylated TA.<sup>34</sup> The degree of alanylation of the TA depends on the species, the growth medium, the pH and the temperature.<sup>6</sup> The TA chains undergo less electrostatic repulsion when alanylated to a higher degree (increased neutrality) and probably by forming stabilizing ion pairs that change the structure and porosity of the cell-envelope.<sup>35</sup> Mutants that lack alanine moieties on the TA and thus have a higher overall negative charge are known to be more susceptible to antimicrobial peptides, antibiotics and lytic enzymes.<sup>36-40</sup> These mutants are more easily targeted by the TLR-2 dependent host defense system and they can be killed using a significantly lower amount of vancomycin.<sup>38</sup> Additionally, these alanine deficient strains show impairment of their ability to bind to hosts or artificial surfaces and, surprisingly, a lower autolysine activity. The functions of glycosyl substitutions on TAs are poorly understood, but it was observed that a genetically altered *Staphylococcus aureus* mutant, lacking the glycosyl transferase necessary for this substitution was significantly less virulent.<sup>41</sup>

## TAs and the immune system

Immunological studies have shown that TAs may activate both the innate as the adaptive immune system.<sup>4-6</sup> The effect on the innate immune system of TA and especially LTA has been studied quite extensively. It is thought that in combination with peptidoglycan TA is the Gram-positive counterpart of lipopolysaccharide (LPS), the most important cause of Gram-negative septic shock. However, whereas LPS activates the innate immune system through binding with Toll-like receptor 4 (TLR-4)<sup>42</sup>, it is not clear how LTA activates the innate immune system.<sup>43</sup> Until recently it was presumed that LTA by virtue of its amphiphilic nature was recognized by TLR-2.<sup>44,45</sup> However, a clear connection between the immune response and LTA has not been established. This can be explained by the fact that the LTA preparations contained immunogenic impurities (lipoproteins, lipids, peptidoglycan) that interfered in the experiments, and/or that the LTA structures were (partly)

decomposed. The D-alanyl esters present on the TA backbone can be easily lost during the purification process. Additionally, TA preparations from biological sources are generally microheterogeneous, which makes it difficult, if not impossible, to perform structure activity relationship studies on these molecules. In 2002, the immunostimulatory activity of LTA was proven when Morath *et al.* observed and quantified the cytokine induction in human whole blood of a fully synthetic staphylococcal LTA (the synthesis of which is described later in this chapter).<sup>46</sup> The question on how the innate immune system is triggered by LTA remains unanswered. In 2006 Hashimoto and Götz impugned the hypothesis that LTA is a ligand of TLR-2 by the finding that a lipopeptide deficient mutant of *S. aureus* showed negative results when tested on TLR-2 dependent activation.<sup>47</sup> These findings were corroborated when in 2009 Kang *et al.* reported on the binding of pneumococcal LTA to TLR-2. Although these researchers were able to obtain a crystal structure from an LTA/TLR-2 complex, binding of LTA to the TLR-2/TLR-6 heterodimer, essential for initiation of the intracellular immune response was not observed.<sup>48</sup> Schmidt and coworkers suggested that innate immune recognition of LTA occurs via the complement system and, specifically, binding of the TA to a lectin.<sup>43,49</sup>

An effect of TAs on the adaptive immune system can be similar to that of bacterial capsular polysaccharides. Opsonophagocytic antibodies directed to these polysaccharides are at the basis of protective immunity against various encapsulated bacteria.<sup>50-56</sup> Like capsular polysaccharides, TAs can be recognized by the immune system of the host as they are exposed on the surface of many Gram-positive bacteria.<sup>52</sup>

*Enterococcus faecalis*, a normally harmless commensal bacterium has become a considerable threat in hospitals as growing antibiotic resistance makes this species difficult to treat. Currently, it is ranked second among the most prevalent Gram-positive nosocomial infection sources and accounts for 10% of all infections in intensive care unit patients, causing septic shock, urinary tract infections, peritonitis and endocarditis. Because of its reduced susceptibility against antibiotics such as vancomycin, the interest in finding alternative remedies is renewed.<sup>57</sup> In 1999 Wang *et al.* described that antiserum against a polysaccharide of *E. faecalis* strain 12030 was able to kill a homologous strain. In addition, the serum was capable of killing various other heterologous strains, including other *E. faecalis* strains and also *E. faecium* strains. Notably, also some vancomycin resistant strains were susceptible to killing by this serum. It was also shown that the purified capsular polysaccharide of *E. faecalis* could be used as an active vaccine against *E. faecalis* infections in mice.<sup>58</sup> In 2006, Theilacker *et al.* reported that the polysaccharide of *E. faecalis* 12030 against which the opsonic antibodies were directed, actually was a type I LTA structure. Notably, this LTA lacked D-alanyl modifications in the backbone.<sup>59</sup> In another study the group of Huebner reported the cross-reactivity of rabbit antibodies raised against *E. faecalis* 12030 LTA. These antibodies were capable of binding heterologous LTA from different enterococcal strains as well as the LTA of *S. aureus* and *S. epidermis* to some

extent. In addition, the antibodies opsonized *S. aureus* and *S. epidermis* and passive immunization with the rabbit serum led to clearance of *E. faecalis* and *S. epidermis* from the bloodstream in mice. These studies indicate that the raised opsonic antibodies were directed at a common structure in the LTA of all these Gram-positive bacteria. Because the LTA structures differ by the nature of the C-2 appendages of these species (different carbohydrate substituents are encountered in different species) the group of Huebner argued that the antibodies were directed against poly-1,3-glycerolphosphate, the backbone of all LTA structures.<sup>60</sup>

## Synthetic strategies to TAs and applications thereof

This part of the introduction describes a selection of syntheses of TAs reported throughout the past 30 years. Since, the crucial linkages in TAs are phosphodiester, the assembly of TAs has benefited from the phosphorylation procedures developed in the field of nucleic acid chemistry and the first part of this section will briefly sum up the main synthetic approaches to the introduction of phosphodiester bonds (see Figure 5).<sup>61</sup>

In the mid 1950s and the early 1960s the groups of Todd and Khorana were leading in the development of methods for the synthesis of DNA and RNA fragments. Although Todd and coworkers were the first to obtain a fully synthetic dinucleotide<sup>62</sup>, Khorana and his coworkers advanced and refined the phosphodiester approach, the first common approach to oligo(deoxy)nucleotides.<sup>63-65</sup> In the key step of this approach a phosphomonoester is made suitable for nucleophilic attack by activation with a condensation agent (e.g. *N,N*-dicyclohexylcarbodiimide, tosyl chloride) to give a transient leaving group, which is displaced by a nucleophile (e.g. a nucleoside or an

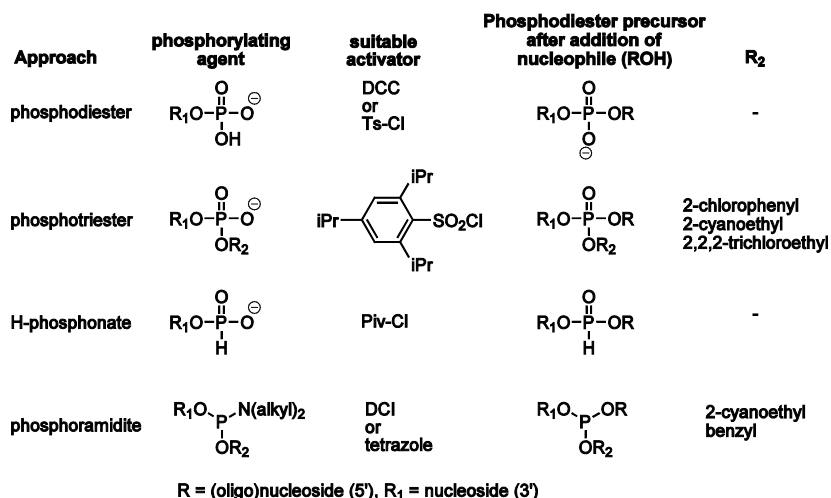


Figure 5. Synthetic approaches that (ultimately) lead to phosphodiester.

oligonucleotide 5' hydroxyl). In this way an unprotected, charged phosphodiester is immediately obtained. The yields obtained by using this approach were generally reasonable (60-70%) in a linear approach, but significantly lower for block-couplings.<sup>66</sup> Several side reactions can occur. The phosphomonoester substrate can be activated twice and the resulting phosphodiesters, although less reactive, are still vulnerable to the activating agents, resulting in symmetric phosphotriesters and pyrophosphates. Notably, an increase in the chain length of the oligonucleotide synthesis is accompanied by an increase of the quantity of unwanted side products. Another major drawback of the phosphodiester approach is the purification procedure to separate the target compound from the often also charged side products. The required ion-exchange chromatography step is a slow and labor-intensive purification procedure. DNA fragments up to a tetramer have been made via this approach before it was abandoned in favor of the development of a more efficient method, the phosphotriester approach. The synthesis of TAs has not been explored with the aid of the phosphodiester approach.<sup>66,67</sup>

The phosphotriester approach is a common term for a wide variety of procedures developed throughout the early 1960s to the late 1980s, especially by the groups of Letsinger<sup>68,69</sup>, Reese<sup>70</sup>, Eckstein<sup>71</sup> and van Boom<sup>72,73</sup>. The conformity of nearly all the phosphotriester procedures is the use of a substituted phenol as phosphate protecting group. In the classical phosphotriester approach a phosphodiester, consisting of a suitably protected nucleoside and a phenol (e.g. 2-chlorophenol) protecting group is made suitable for nucleophilic attack by reaction with an activating agent (e.g. triisopropylphenylsulfonyl chloride) to give an activated intermediate that rapidly reacts with an alcohol of choice (e.g. a second suitably protected nucleoside) under basic conditions to produce the target compound as a phenol protected phosphotriester. Compared with the phosphodiester method, phosphotriester approaches generally have shorter reaction times, result in higher coupling yields (especially in block-couplings) and are purified by less laborious chromatographic procedures due to the uncharged phosphotriester intermediates. However, also the phosphotriester approach suffers from the occurrence of side reactions. For example, even the use of sterically compromised activating agents (e.g. triisopropylphenylsulfonyl chloride) does not prevent unwanted sulfonylation of the incoming alcohol in the condensation step. In the synthesis of oligonucleotides the reaction time increases and the yield decreases with the growing of the oligonucleotide chain. Finally, the removal of the phenol protecting groups in the final stage of the synthesis can be accompanied by unwanted phosphate cleavage reactions.<sup>61</sup>

Todd and co-workers<sup>74</sup> already reported on the synthesis of a dinucleotide using a H-phosphonate intermediate in the late 1950s and renewed interest in the H-phosphonate approach was initiated in 1985 by the group of Garegg which was continued by this and several other research groups for the next ten years.<sup>75</sup> In the condensation step of this approach, activation of an H-phosphonate monoester with a reagent such as pivaloyl chloride results in an activated intermediate, which reacts

with an alcohol to give a H-phosphonate diester. Oxidation of this H-phosphonate diester directly leads to the target phosphodiester. The H-phosphonate approach is especially suitable for syntheses on solid support and the elongation sequence of an automated DNA synthesis protocol consists of only two steps: coupling and liberation of the 5' hydroxyl. At the final stage of the synthesis, global oxidation and deprotection affords the target oligonucleotide. The coupling yields are generally high (>90%) and oligonucleotides of a length of more than hundred residues have been successfully made.<sup>76-78</sup> Shortcomings of this method are that during the coupling step side reactions can occur by over-activation of the H-phosphonate mono- and diesters, and also that H-phosphonate diesters are prone to transesterification. These drawbacks and favorable properties of the phosphoramidite method (see below) are the reason for the limited use of the H-phosphonate approach in contemporary oligonucleotide synthesis.<sup>79,80</sup>

The most effective and widely used method to obtain phosphodiesters is the phosphoramidite approach, introduced by Beaucage and Caruthers in the early 1980s.<sup>81</sup> The decisive advantage of this approach is the optimal reactivity of the intermediates at room temperature and the selectivity of the reactions, leading to a minimum amount of by-products. This method was derived from the earlier developed, less widely used, phosphite triester approach, in which chloride is used as a leaving group. The main disadvantages of the phosphite triester approach are the practical difficulty (reaction temperatures around -80 °C) and the fact that it leads to a number of by-products.<sup>82</sup> In the phosphoramidite approach the chlorine as leaving group is replaced by a dialkyl amino moiety, which can be activated by mild and non-nucleophilic acids such as tetrazole ( $pK_a$  4.9), to allow substitution by an alcohol. In the next step the obtained phosphite triester is oxidized to give the more stable phosphotriester. Soon after the introduction of this approach, it was found that the *N,N*-diisopropyl phosphoramidites are most suitable for oligonucleotide synthesis as these amidites are considerably more stable than the corresponding *N,N*-dimethyl derivatives and have a half-time of ~200 hrs in aqueous acetonitrile (at 25 °C).<sup>83-85</sup> The stability of the phosphoramidites in combination with the short reaction times (normally within seconds) with alcohols upon activation with a weak acid as well as the fact that no significant side-reactions take place during the elongation sequence are the main advantages of this approach.<sup>61,86</sup>

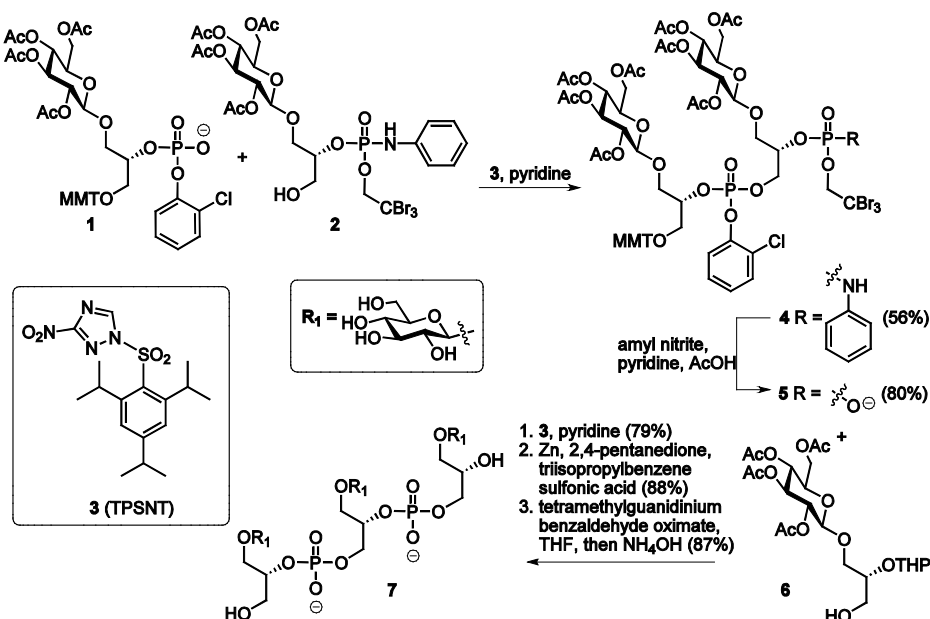
The past decades, besides oligo(deoxy)nucleotides, various phosphodiester containing (bio)molecules have been synthesized using the approaches described above. A salient example is the antigenic capsular polysaccharide from *Haemophilus influenza*.<sup>87,88</sup> In the framework of the development of a vaccine, several research groups explored the synthesis of functionalized fragments of this polysaccharide (see Scheme 1). Oligomers with ribosylribitol phosphate as repeating unit, have been prepared via a solution phase modified phosphotriester approach (Van Boom)<sup>89,90</sup>, an automated solid phase H-phosphonate approach (Nilsson)<sup>91</sup>, solution phase/soluble support phase phosphoramidite approaches (Van Boom, Klein)<sup>92,93</sup> and a controlled





excluding the formation of a cyclic phosphate in the “middle” unit. Therefore, phosphodiester (**1**) was coupled to tribromoethylphosphor anilidate (**2**) using triisopropylphenylsulfonyl nitrotriazolate (TPSNT, **3**) as the condensating agent.<sup>96</sup> The phosphoramidate proved stable under the coupling conditions, the TPSNT reagent gave minimal unwanted alcohol sulfonylation and the dimer (**4**) was obtained in 56%. The aniline moiety was cleaved in a Sandmeyer type reaction using amyl nitrite in a mixture of AcOH and pyridine to give phosphodiester **5** in 80% yield.<sup>97</sup> Phosphodiester **5** was coupled with 2-*O*-THP protected glucosyl glycerol building block **6** again under influence of TPSNT to give the protected trimer in 79%. A four-step and two-pot deprotection procedure finally gave the deprotected TA trimer **7** in good yield (~75%).<sup>95</sup>

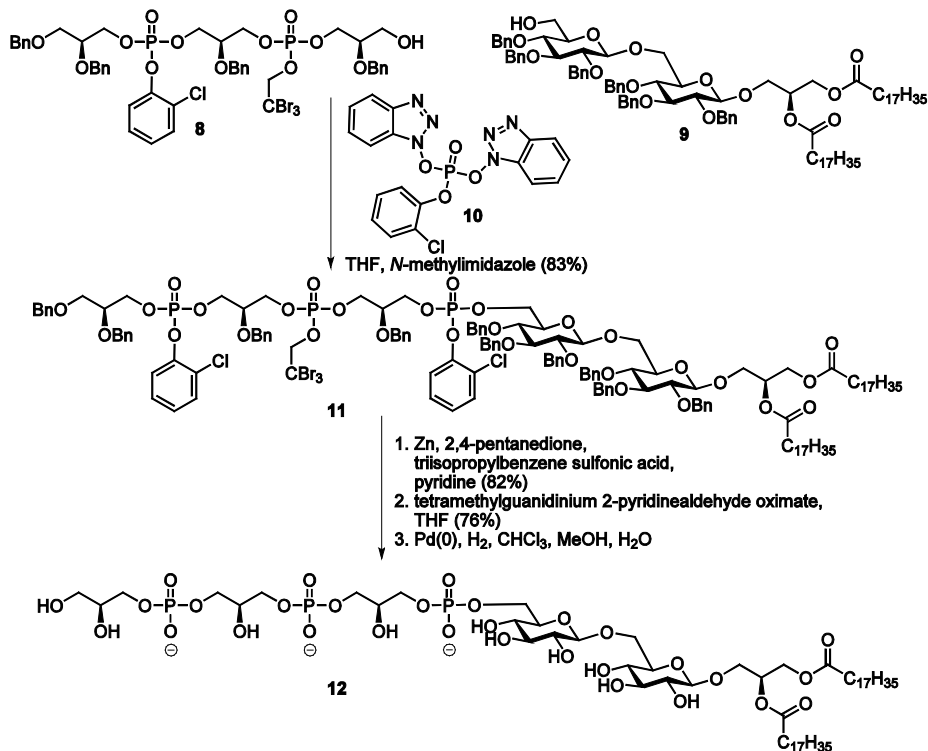
**Scheme 2.** Synthesis (phosphotriester approach) of van Boeckel *et al.* to a *B. subtilis* TA trimer.



In 1982 Oltvoort *et al.* reported on the synthesis of the gentiobiosyl diacylglycerol glycolipid anchor of *Staphylococcus aureus* LTA.<sup>98</sup> Two years later, the same group of researchers completed the convergent synthesis of an elongated fragment, consisting of the same glycolipid anchor connected to a glycerol phosphate trimer (see Scheme 3).<sup>99</sup> The protected glycerol phosphate trimer (**8**) was obtained via a multistep strategy in which the phosphotriester functions were introduced in high yield by the use of the bifunctional phosphorylating agents 2-chlorophenyl-*bis*-benzotriazole phosphate **10**<sup>73</sup> and the corresponding 2,2,2-tribromoethyl agent (not shown). Block coupling of trimer **8** with benzylated gentiobiosyl alcohol **9** using phosphorylating agent **10** gave the protected construct **11** in 83% yield. The deprotection procedure consisted of the following three steps: 1) removal of the tribromoethyl moiety using activated zinc, 2) hydrolysis of the 2-chlorophenylphosphate triesters using

tetramethylguanidinium 2-pyridinylaldehyde oximate, and 3) hydrogenolysis of the benzyl ethers, giving LTA fragment **12**. The first two steps proceeded uneventfully in ~60% while the yield of the latter step is not mentioned.

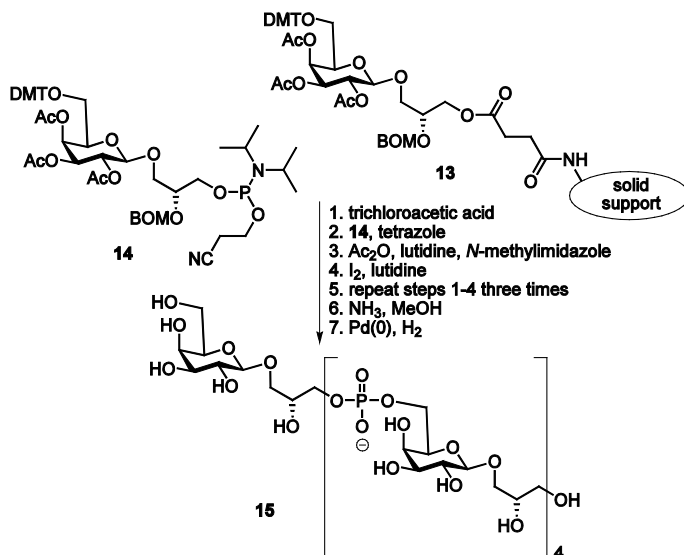
**Scheme 3.** Oltvoort's synthesis of *S. Aureus* lipoteichoic acid carrier fragment.



The third TA fragment synthesized by the group of Van Boom features the type II teichoic acid from *Bacillus licheniformis* ATCC 9945, which consists of a 1-O-(β-D-galactopyranosyl)glycerol repeating unit (see Scheme 4). A pentameric fragment of this TA was synthesized using an automated solid phase procedure in combination with phosphoramidite chemistry.<sup>100</sup> Immobilized **13** and phosphoramidite **14** were both obtained from the same orthogonally protected galactosyl glycerol building block. With the aid of a DNA/RNA synthesizer, resin **13** (200 mg, 10 μmol) was subjected to the following sequence of reactions: 1) 2% trichloroacetic acid in DCM (removal of the DMT ether), 2) phosphoramidite **14** and tetrazole in MeCN (elongation step), 3) Ac<sub>2</sub>O, 2,6-lutidine and *N*-methylimidazole (capping of remaining unreacted alcohols), and 4) I<sub>2</sub> in 2,6-lutidine and 1,4-dioxane (oxidation of the phosphite intermediate). After a three-fold repetition of this elongation cycle, the resin was removed from the synthesizer and treated with methanolic ammonia to cleave the base labile acetyl and cyanoethyl groups and to release the TA fragment from the resin. Finally, purification using size exclusion chromatography and subsequent hydrogenolysis afforded target pentamer **15** in good yield (~25% from

immobilized **13**). It was described that for efficient couplings 50 equivalents of phosphoramidite **14** were needed. Nonetheless, this work presented a significant improvement compared to the previously described syntheses in terms of time and labour efficiency. Additionally, the chemistry (phosphoramidites) and methodology (DNA synthesizer) that were amended were at the time both still in its infancy. Surprisingly, no more TA fragments were synthesized in the following years using this seemingly suitable technique.

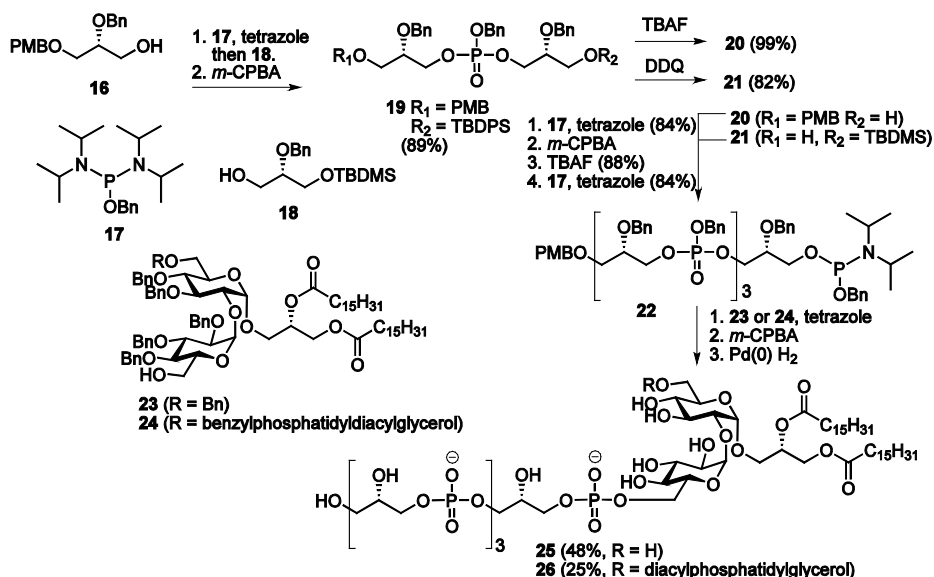
**Scheme 4.** Westerduin's automated solid phase approach to a *B. Licheniformis* TA pentamer (**15**).



In the early 1990s Fukase *et al.* reported on the syntheses of LTA fragments derived from *Streptococcus pyogenes* and *Enterococcus hirae*.<sup>101,102</sup> The closely related structures contain a 1-(diacylglycerol)- $\alpha$ -D-kojibiosyl (2-*O*-[ $\alpha$ -glucosyl]-glucosyl) glycolipid with the polyglycerol phosphate backbone linked to the C-6 of the terminal glucosyl moiety. The LTA of *E. hirae* LTA contains an additional phosphatidyl diacylglycerol part, which is connected to C6 of the inner glucosyl (see Scheme 5). In the synthetic targets of Fukase *et al.* the naturally occurring oleoyl esters were replaced by palmitoyl tails and the glycerol phosphate backbone, normally comprising ~20 subunits was brought back to a tetrameric unit. As can be seen in Scheme 5 the glycerol phosphate part was assembled via a convergent approach. Starting from an enantiomerically pure glycerol derivative, two differently and orthogonally protected building blocks (**16** and **18**) were acquired and connected via phosphoramidite chemistry comprising phosphitylation, tetrazole mediated coupling and oxidation. The resulting dimer (**19**) was treated with either DDQ or TBAF, resulting in alcohols **20** and **21**, respectively, which were connected using the sequence of reactions mentioned above. The TBDMS in the tetrameric fragment was cleaved with TBAF and the resulting alcohol was converted into the benzylamidite (**22**) using benzyl bis-(*N,N*-

diisopropyl)phosphordiamidite and tetrazole. Finally, the glycolipid anchor (**23** or **24**) was coupled to the TA phosphoramidite (**22**) under the agency of tetrazole to give, after deprotection, the respective target TA fragments **25** and **26**. The coupling, oxidation and single step deprotection in case of *S. pyogenes* LTA (**25**) proceeded uneventfully in 48% yield over three steps. In the final coupling and oxidation of **22** and **24** the initial product was obtained in a significantly lower yield (27%). In the latter case a severely lowered reaction concentration (~65 mM for **23** vs ~5 mM for **24**) was employed, suggesting that the bis(diacylglycerol) glycolipid is less soluble. The final deprotection of the more complex **26** proceeded in high yield (93%). Both fragments were evaluated for their ability to induce cytokine production in muramyl dipeptide primed mice, but no activity was detected.<sup>103</sup>

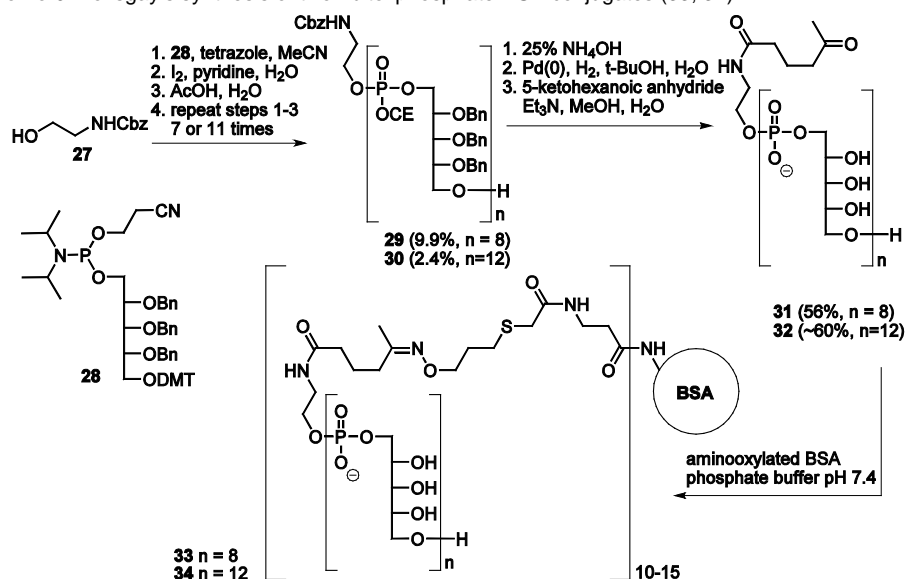
**Scheme 5.** Phosphoramidite approach by Fukase *et al.* to proposed LTA carrier structures of *S. pyogenes* (**25**) and *E. Hirae* (**26**).



Poly-(1,5-ribitol phosphate) is ubiquitously found in the WTA of Staphylococci and Bacilli. In 2006, the group of Pozsgay reported on the synthesis of an octa- and a dodecamer ribitol phosphate (**33** and **34**) and their subsequent conjugation to bovine serum albumin (BSA).<sup>104</sup> Their first attempt to make a hexameric fragment of these WTA oligomers comprised an automated solid phase approach using phosphoramidite **28**. However, an intractable mixture, in which a hexamer predominated, was obtained and the applied chemistry was reinvestigated in a solution phase approach using aminospacer alcohol **27** as starting compound. Coupling of **27** with phosphoramidite **28** under influence of tetrazole, subsequent oxidation of the phosphite, and detritylation with 2% trichloroacetic acid in DCM, reportedly, resulted in loss of the integrity of the phosphotriester moieties. Switching to the milder AcOH/water/DCM (85:10:5) detritylation cocktail resulted in selective

cleavage of the DMT without loss of the phosphotriesters. However, the prolonged reaction time (four hours instead of a few minutes for TCA/DCM) prompted to abandon the automated solid phase approach and continue their synthesis in solution (see Scheme 6). Using this approach fully protected octamer **29** was obtained in 9.9% yield over 8 elongation cycles/24 steps (~75% per cycle consisting of coupling, oxidation, detritylation) and dodecamer **30** was obtained in 2.4% yield over 12 elongation cycles/36 steps (~73 % per cycle). Release of the cyanoethyl groups was accomplished by  $\beta$ -elimination using ammonium hydroxide in a mixture of methanol and water. The subsequent hydrogenolysis proceeded uneventfully after the EtOH/H<sub>2</sub>O mixture was replaced by *t*BuOH/H<sub>2</sub>O as the former combination resulted in partial *N*-ethylation. The fully deprotected oligomers were treated with 5-ketohexanoic anhydride and the resulting *N*-acylated octamer **31** (56% over three steps) and dodecamer **32** (~60%) reacted with aminooxylated BSA<sup>105</sup> forming a stable oxime linkage. The evaluation of the immunogenic properties of these conjugates, which contained 10-15 WTA fragments per molecule of BSA, has not been reported in the literature so far.

**Scheme 6.** Pozsgay's synthesis of two ribitol phosphate-BSA conjugates (**33**, **34**).

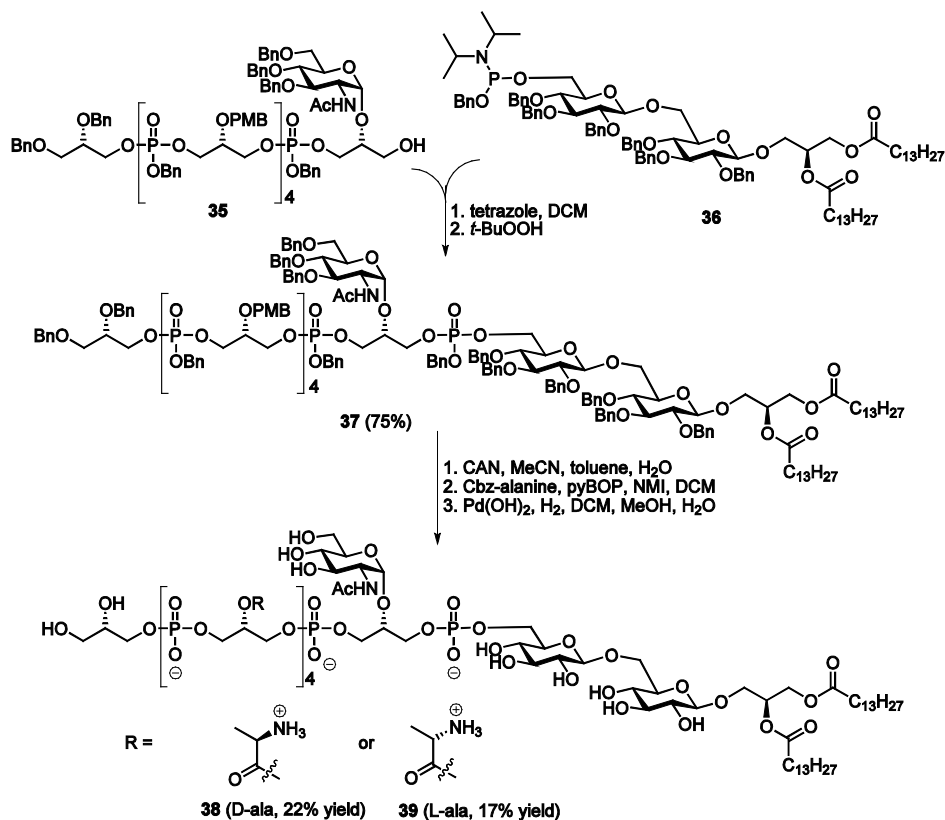


About a decade ago, the group of Hartung developed an improved method for the isolation and purification of LTA from *Staphylococcus aureus*, which involved a butanol extraction followed by hydrophobic interaction chromatography (HIC) using a slightly acidic buffer (NH<sub>4</sub>OAc, pH 4.7). With this method it was ensured that the D-alanine esters present on the polyglycerol phosphate backbone were preserved and the structure of the full LTA could be revised: The gentiobiosyl glycerol glycolipid anchor, containing a mixture of saturated fatty acid tails (~C16), is linked via the C6 hydroxyl of the sugar to the glycerol phosphate backbone. The glycerol phosphate

part, averaging 45-50 residues in length was found substituted with D-alanine esters (~70%) and  $\alpha$ -D-N-acetylglucosamine (~15%). A small part of the TA was found unsubstituted (~15%).<sup>14</sup>

In 2003, the group of Schmidt reported on the synthesis of this revised LTA structure. Their first aim was to make an LTA construct, containing a glycerolphosphate backbone with a glycerol C-2 substitution pattern similar to the native staphylococcal LTA. Target LTA **38** (see Scheme 7) consists of a glycolipid moiety connected to a hexaglycerol phosphate provided with four D-alanine esters and one N-acetylglucosaminyl residue.<sup>106</sup> To postpone the installation of the labile alanine esters to the penultimate stage of the synthesis the temporary *para*-methoxybenzyl (PMB) protecting group at the glycerol C-2 was applied. It was reasoned that the PMB groups could selectively be cleaved in the fully protected stage of the LTA construct, followed by introduction of the alanine esters. Starting from dibenzylglycerol the hexaglycerol phosphate backbone (**35**) was made in a linear, step-wise procedure (elongation, oxidation, desilylation) using either the 2-*O*-PMB or 2-*O*-(N-acetyl- $\alpha$ -glucosaminyl) glycerol benzylphosphoramidites. After a block coupling of hexameric alcohol (**35**) and the glycolipid anchor benzylphosphoramidite (**36**) under the agency of tetrazole followed by oxidation, fully protected **37** was obtained in 75% yield. The PMB ethers were replaced by protected alanine esters by treatment of **37** with cerium ammonium nitrate (67% yield) and esterification with N-carbobenzyloxy-D-alanine using PyBOP as the condensing agent (70% yield). Finally, the protected precursor was deprotected in a single step ( $\text{Pd}(\text{OH})_2/\text{H}_2$ ) and purified with hydrophobic interaction chromatography giving LTA **38** in 47% yield. In addition, in an analogous manner LTA **39**, equipped with L-alanine esters was prepared (62% yield, Scheme 7). The immunostimulatory properties of both LTA fragments were assessed by an assay in which the response of human blood leukocytes was determined. This assay revealed that LTA **38** has a similar effect on cytokine production as the LTA which was isolated by the group of Hartung. Contrary, L-alanylated LTA **39** showed a strongly diminished activity (10-100 fold) compared to the D-alanylated fragment, indicating that alanine modifications play a critical role in recognition by the immune system of hosts.<sup>14,46</sup>

With the objective to determine the minimum structural properties required for innate immune response the group of Schmidt continued their research by synthesizing a number of *S. aureus* LTA derivatives. A convergent approach was devised that made it possible to prepare several constructs by minor alterations of the synthetic route. In 2005, the syntheses of fragments **40** and **41** were described (see Scheme 8).<sup>107</sup> Whereas LTA **40** only lacked the gentiobiosyl moiety, derivative **41** features more radical changes: both gentiobiosyl and GlcNAc moieties were omitted and the D-alanine esters were replaced by the more stable amide analogues on the C-2 glycerol residues of opposed stereochemistry. The effects of these modifications on the immunostimulatory properties were minimal, showing that the gentiobiosyl and

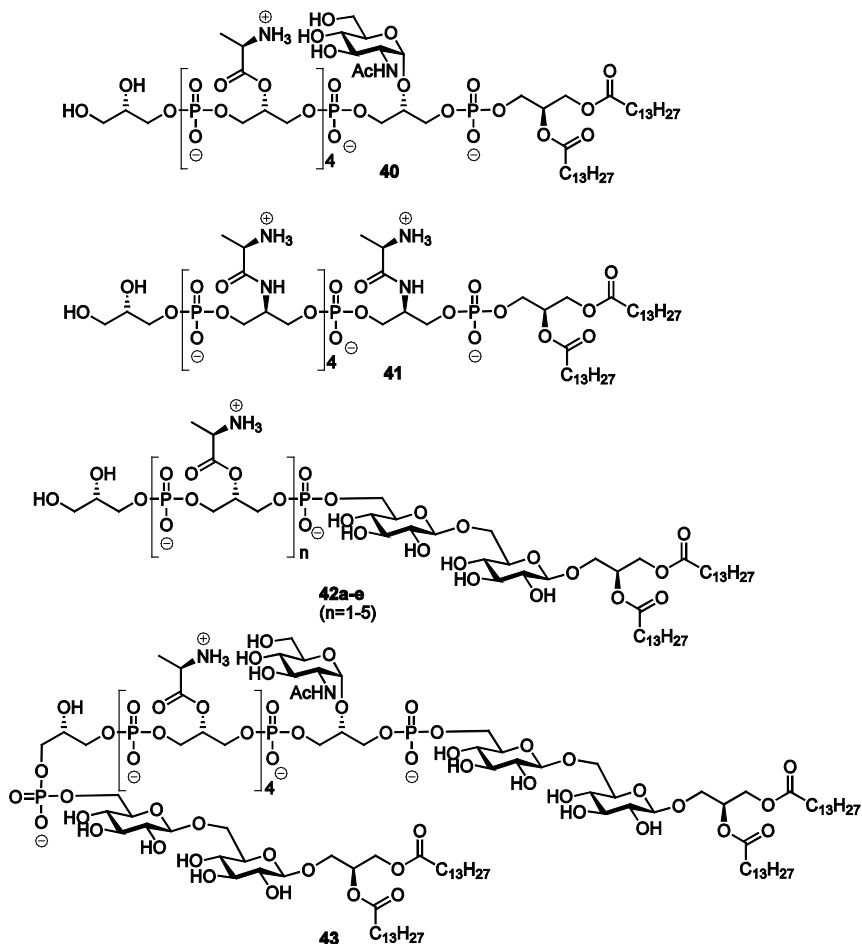
**Scheme 7.** Schmidt's synthesis of a complete staphylococcal LTA construct (**38**) and analogon (**39**).

GlcNAc were not necessary for cytokine (IL-8, TNF- $\alpha$ ) production in human whole blood, peritoneal macrophages or TLR-transfected HEK-cells.<sup>108</sup> The presence of the alanylated glycerol phosphate backbone proved more important. This finding was confirmed shortly thereafter by the synthesis and evaluation of TA derivatives **42a-e** by the same authors. These compounds consist of the complete gentiobiosyl diacyl glycerol moiety connected to an oligoglycerol phosphate backbone ranging in length between two (**42a**) and six residues (**42e**) and containing between one and five alanine modifications (Scheme 8).<sup>109</sup> Biological evaluation showed that a minimum of two alanylated glycerol phosphates in the construct is required for significant activity (10-fold increase of cytokine induction compared to one alanylated residue). The gentiobiosyl diacyl glycerol anchor (structure not shown) alone did not induce any immune response. However, it was suggested that the presence of this moiety in LTA derivatives is important for innate immune activity. The group of Schmidt also reported the synthesis of a construct that contained all the natural substitutions and a second glycolipid moiety (structure **43**, Scheme 8).<sup>110</sup> It was shown that in an immune assay cytokine (IL-8, TNF- $\alpha$ ) titers were significantly higher compared to the monoglycolipid **38** (see Scheme 7). The presentation of the crucial recognition



elements, such as the D-alanyl esters, could possibly be increased by this bis-amphiphilic construct.<sup>43,110</sup>

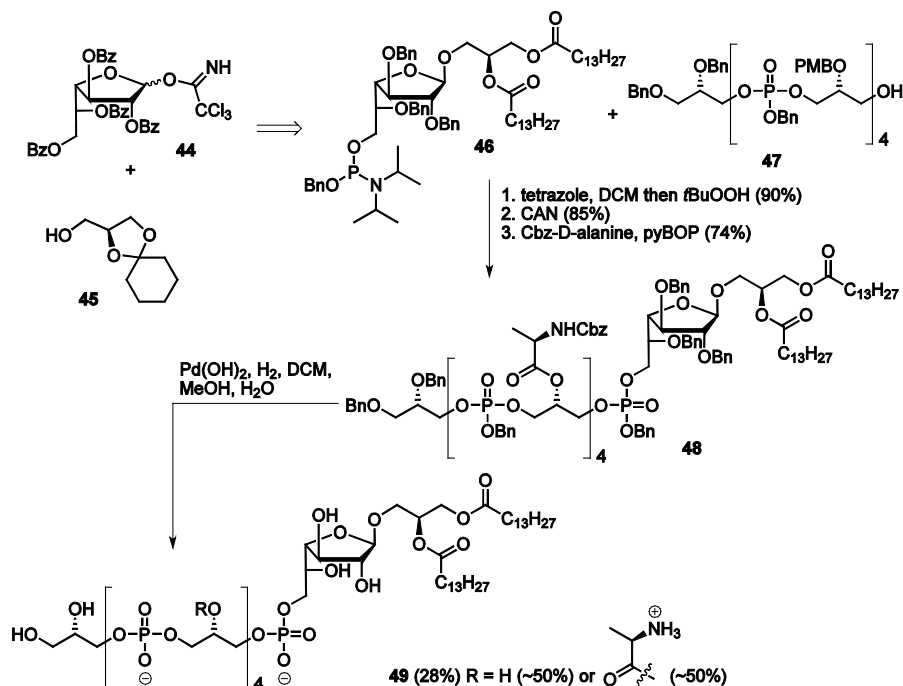
**Scheme 8.** Synthetic derivatives of *Staphylococcus aureus* LTA (**40-43**) made in the lab of Schmidt.



The type I LTA from *Streptococcus* sp DSM 8747, a mutant which is genetically related to *Streptococcus pneumoniae*, consists of a galactofuranoside diacyl glycerol glycolipid with the oligoglycerol phosphate backbone connected to the C-6 of the galactofuranose residue. The backbone is substituted for about 30% at C-2 positions with D-alanine esters.<sup>111</sup> In 2010, Schmidt and coworkers reported on the synthesis of a fragment of this LTA.<sup>112</sup> The  $\beta$ -galactofuranoside diacylglycerol phosphoramidite **46** was constructed via the imidate glycosylation procedure using anchimeric assistance of a temporary 2'- benzoyl ester, followed by several consecutive (protecting group) manipulations (see Scheme 9). Block coupling of the previously reported PMB-protected pentamer **47** and glycolipid phosphoramidite **46** using tetrazole followed by oxidation yielded the fully protected intermediate in 90%. At this stage the PMB

ethers were selectively cleaved (85% yield) and the resulting tetraol was decorated with alanyl moieties using Cbz-D-alanine and PyBOP (74% yield). Global deprotection of the fully protected **48** (containing four alanine esters) followed by purification resulted in the target LTA fragment **49** provided with roughly two alanine esters. It is unclear why the alanine esters in this particular molecule were prone to hydrolysis, as this phenomenon was not observed in similar syntheses reported earlier by the group of Schmidt.<sup>106,107,109,110</sup>

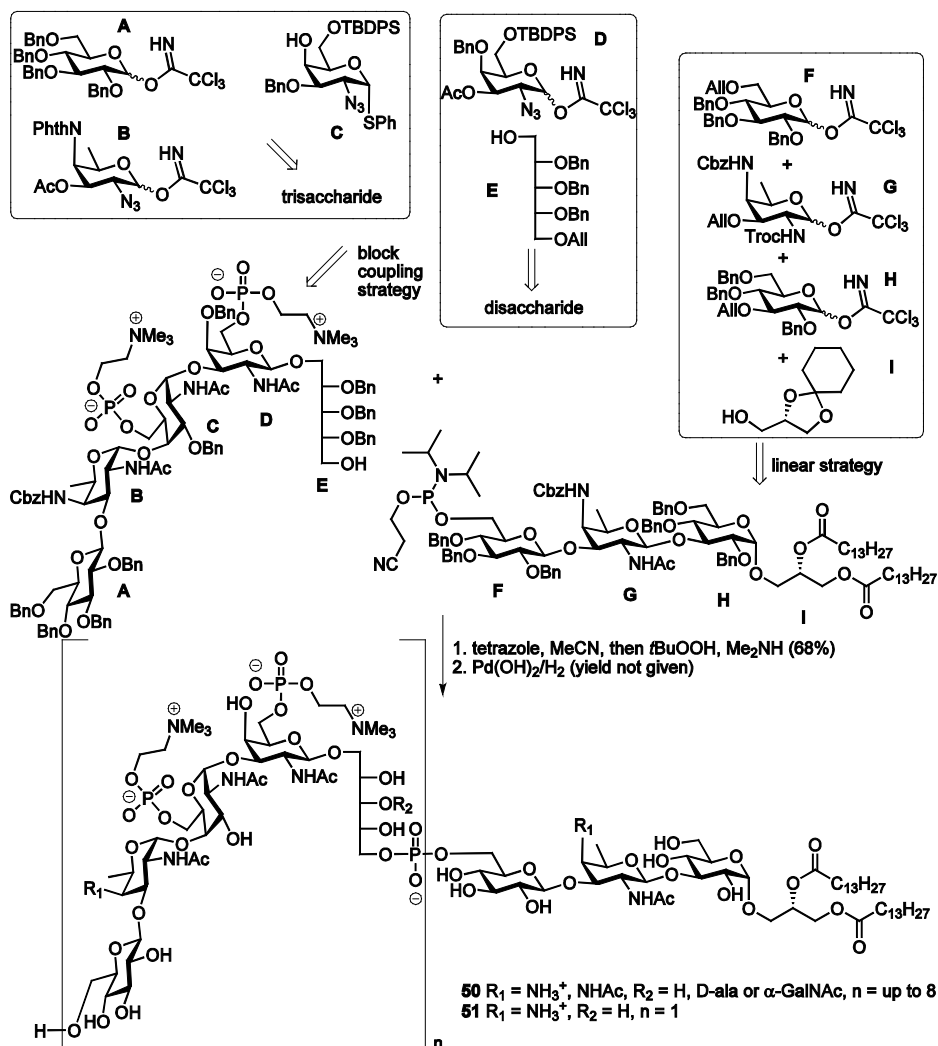
**Scheme 9.** Synthesis of the unusual LTA as found in *Streptococcus* species DSM 8747.



The complex structure of the type IV LTA found in *Streptococcus pneumoniae* is made up from a glycolipid anchor, that consists of diacylglycerol linked to a trisaccharide (glucose-diaminofucose-glucose), connected to a repeating unit ( $n = 1-8$ , 2 on average) pentasaccharide phosphate (glucose-diaminofucose-[phosphatidylcholine]-galactosamine-[phosphatidylcholine]galactosamine-ribitol) (see Scheme 10, structure **50**).<sup>113</sup> In 2010, Pedersen *et al.* reported on the synthesis of the structural variant **51** in which the diaminofucose moieties were mono-(2')-N-acetylated and the ribitol unit was non-substituted.<sup>114</sup> The synthesis comprised 88 steps starting from the protected monosaccharide building-blocks. The pentasaccharide repeating unit was constructed by the coupling of trisaccharide **ABC** and disaccharide **DE**. Ensuing manipulations (reduction of the azides, acetylation and HF/pyridine treatment) was followed by decoration of the resulting alcohols on subunits **C/D** in the pentasaccharide with phosphatidylcholines. Next, the terminal alcohol of the ribitol (**E**) was selectively unmasked by isomerization of the allyl ether

and cleavage of the resulting 1-propenylether, giving pentasaccharide **ABCDE** (see Scheme 10). The glycolipid phosphoramidite (**FGHI**) was constructed via a linear glycosylation strategy. First alcohol **I** was glycosylated using imidate **H** and, subsequently, the cyclohexylidene group was removed. The resulting diol was myristoylated and after several protecting group manipulations, glycosylations and finally phosphitylation, the phosphoramidite was acquired. The crucial step was the coupling of pentasaccharide alcohol **ABCDE** (including zwitterionic phosphatidylcholine functionalities) and glycolipid phosphoramidite **FGHI**. Phosphitylation in acetonitrile under influence of the activator tetrazole and oxidation under basic conditions, by which the cyanoethyl phosphate protecting groups were

**Scheme 10** *Streptococcus pneumoniae* LTA (**50**). Structure **51** was synthesized by Pedersen *et al.*



concomitantly cleaved, the partially protected pseudononasaccharide triphosphate (**ABCDEFGHI**) was obtained in 68% yield. Finally, global deprotection ( $\text{Pd}(\text{OH})_2/\text{H}_2$ ) gave the target LTA construct (**51**).

At the same time the separate deprotection of the glycolipid<sup>115</sup> and repeating unit<sup>116</sup> moieties were undertaken. The resulting compounds (**51**, glycolipid and repeating unit) were evaluated on their potency to activate the innate immune system in human whole blood cells and isolated human mononuclear cells. It was found that both the sole glycolipid and construct **51** stimulated the release of the pro-inflammatory cytokine IL-8. The repeating unit alone did not result in any innate immune response. These results support the hypothesis that LTA fragments are not recognized by TLR-2 or 4. According to the authors these molecules most probably activate the innate immune system via the complement system and, more specifically, are recognized via the lectin pathway of the complement system.<sup>114-116</sup>

## Conclusion

TAs are important Gram-positive bacteria cell wall components. Although TAs are structurally diverse and microheterogenous, their basic structure consists of alditol phosphates, randomly decorated with sugars and alanine esters. TAs are ligands for both the innate and the adaptive immune system. In this respect organic synthesis is indispensable to obtain well-defined TA fragments of desired length and substitution pattern. Subsequent structure activity relationship studies of these fragments may provide insights in mechanism of the immune system and help in the development of future vaccines.

## Outline of this thesis

The work described in this thesis involves the development of synthetic methodologies that give access to glycerol TAs differing in length and composition. Solution, solid phase and fluorous phase chemistry methods are applied to obtain the desired (library of) TA structures, which have been evaluated for their ability to inhibit opsonic killing of *Enterococcus faecalis* by antibodies raised in rabbits against natural LTA of this species. The most active fragments have been coupled to an immunogenic carrier protein and the resulting conjugates evaluated on their immunogenicity and their potency to function as a (Gram-positive) vaccine.

**Chapter two** describes the solution phase synthesis of a glycerol phosphate hexamer, using phosphoramidite chemistry. First, the route towards the suitably protected glycerol phosphoramidite building blocks is presented after which the construction and deprotection of the target oligomer, containing an  $\alpha$ -kojibiosyl ( $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 2)-D-glucose) substituent, is given.

Based on the chemistry presented in chapter two, the assembly of a small library of TAs is described in **chapter three**. These molecules were made with the use of an automated solid phase strategy and a DNA synthesizer. Four fragments ranging in

length from 6<20 glycerol phosphate subunits and two hexamers provided with an  $\alpha$ -glucosyl substitution were synthesized and evaluated on their antigenicity by an opsonophagocytic inhibition assay (OPIA).

**Chapter four** deals with a fluoruous phase protocol for the generation of several (aminoglucosylated) TAs that are found in a range of bacterial species. The protocol combines the advantages of both solution and solid phase strategies, especially through a rapid purification step, using a fluoruous solid phase extraction (F-SPE) methodology after each elongation cycle. These TA fragments, featuring a terminal phosphate moiety are immunologically evaluated.

In **chapter five** the use of an alternative base-labile fluoruous linker is described. The applied succinyl type of linker gives access to non-phosphorylated TA fragments. Additionally, an acid-stable tetrabenzylglucosyl glycerol phosphoramidite building block, that is suitable for incorporation in an earlier stage of the TA oligomer synthesis, is prepared.

**Chapter six** covers the conjugation to an immunogenic carrier protein of the most antigenic TA fragment, the synthesis and evaluation of which is described in chapter three and five. The conjugate is evaluated for its potential to function as a vaccine modality against *E. faecalis* and some other Gram-positive strains. The immunological experiments include: opsonophagocytic assays (OPAs) and passive immunization in a rat endocarditis model using rabbit anti serum raised against the synthetic conjugate.

The work described in **chapter six** has been carried out in cooperation with the Division of Infectious Diseases from the Department of Medicine of the University Medical Center Freiburg: A. Kropec-Huebner, D. Laverde Gomez, C. Theilacker and J. Huebner.

**Chapter seven** consists of a summary of the work described in this thesis. Several synthetic outlooks are given, including improved automated solid phase protocols using a photocleavable linker and a fluoruous tag. Other future prospects include the synthesis of stable D-alanine ester analogues and the synthesis of an improved, clinically relevant, TA-tetanus toxoid conjugate.

## References and footnotes

1. Baddiley, J.; Buchanan, J.G.; Greenberg, G.R. *Biochem. J.* **1957**, *66*, 51-62P.
2. Fischer, W. *Adv. Microb. Physiol.* **1988**, *29*, 233-302.
3. Naumova, I.B.; Shashkov, A.S.; Tul'skaya, E.M.; Streshinskaya, G.M.; Kozlova, Y.I.; Potekhina, N.V.; Evtushenko, L.I.; Stackebrandt, E. *FEMS Microbiol. Rev.* **2001**, *25*, 269-283.
4. Neuhaus, F.C.; Baddiley, J. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 686-723.
5. Weidenmaier, C.; Peschel, A. *Nat. Rev. Microbiol.* **2008**, *6*, 276-287.
6. Swoboda, J.G.; Campbell, J.; Meredith, T.C.; Walker, S. *ChemBioChem* **2010**, *11*, 35-45.
7. Leloir, L.F. *Arch. Biochem. Biophys.* **1951**, *33*, 186-190.
8. Kennedy, E.P.; Weiss, S.B. *J. Biol. Chem.* **1956**, *222*, 193-214.
9. Mitchell, P.; Moyle, J.J. *Gen. Microbiol.* **1951**, *5*, 981-992.
10. Armstrong, J.J.; Baddiley, J.; Buchanan, J.G.; Carss, B.; Greenberg, G.R. *J. Chem. Soc.* **1958**, 4344-4354.
11. Baddiley, J. *Essays Biochem.* **1972**, *8*, 35-77.
12. Archibald, A.R. *Adv. Microb. Physiol.* **1974**, *11*, 53-95.
13. Baddiley, J. *BioEssays* **1989**, *10*, 207-210.
14. Morath, S.; Geyer, A.; Hartung, T. *J. Exp. Med.* **2001**, *193*, 393-397.
15. Kelemen M.V.; Baddiley, J. *Biochem. J.* **1961**, *80*, 246-254.
16. Hay, J.B.; Wicken, A.J.; Baddiley, J. *Biochim. Biophys. Acta* **1963**, *71*, 188-190.
17. Fischer, W. *Handbook of Lipid Research 6. Glycolipids, Phosphoglycolipids and Sulfoglycolipids*; Eds: M. Kates, Plenum Press: New York, USA, **1990**.
18. De Boer, W.R.; Wouters, J.T.M.; Anderson, A.J.; Archibald, A.R. *Eur. J. Biochem.* **1978**, *85*, 433-436.
19. Endl, J.; Seidl, H.P.; Fiedler, F.; Schleifer, K.H. *Arch. Microbiol.* **1983**, *135*, 215-223.
20. Tul'skaya, E.M.; Streshinskaya, G.M.; Naumova, I.B.; Shashkov, A.S.; Terekova, L.P. *Arch. Microbiol.* **1993**, *160*, 299-305.
21. Shashkov, A.S.; Streshinskaya, G.M.; Kozlova, Y.I.; Potekhina, N.V.; Evtushenko, L.I.; Taran, V.V.; Naumova, I.B. *Biochemistry (Moscow)* **1997**, *62*, 1135-1139.
22. Oku, Y.; Kurokawa, K.; Matsuo, M.; Yamada, S.; Lee, B.L.; Sekimizu, K. *J. Bacteriol.* **2009**, *191*, 141-151.
23. Schirner, K.; Marles-Wright, J.; Lewis, R.; Errington, J. *EMBO J.* **2009**, *28*, 830-842.
24. D'Elia, M.; Millar, K.; Beveridge, T.; Brown, E. *J. Bacteriol.* **2006**, *188*, 8313-8316.
25. Kaito, C.; Sekimizu, K. *J. Bacteriol.* **2007**, *189*, 2553-2557.
26. Vergara-Irigaray, M.; Maira-Litran, T.; Merino, N.; Pier, G.B.; Penades, J.R.; Lasa, I. *Microbiol.* **2008**, *154*, 865-877.
27. Fedtke, I.; Mader, D.; Kohler, T.; Moll, H.; Nicholson, G.; Biswas, R.; Henseler, K.; Götz, F.; Zähringer, U.; Peschel, A. *Mol. Microbiol.* **2007**, *65*, 1078-1091.
28. Baddiley, J. *Acc. Chem. Res.* **1970**, *3*, 98-105.
29. Ellwood, D.C. *Biochem. J.* **1970**, *118*, 367-373.
30. Bierbaum, G.; Sahl H.G. *J. Bacteriol.* **1987**, *169*, 5452-5458.
31. Li, M.; Lai, Y.; Villaruz, A.E.; Cha, D.J.; Sturdevant, D.E.; Otto, M. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 9469-9474.
32. Archibald, A.R. *Receptors and Recognition, series B, vol. 7. Virus receptors*; Eds: L.L. Randall, L. Philipson, Chapman & Hall, London, UK, **1980**.
33. Peschel, A.; Vuong, C.; Otto, M.; Götz, F. *Antimicrob. Agents Chemother.* **2000**, *44*, 2845-2847.
34. Heptinstall, S.; Archibald, A.R.; Baddiley, J. *Nature* **1970**, *225*, 519-521.

35. Wickham, J.R.; Halye, J.L.; Kashtanov, S.; Khandogin, J.; Rice, C.V. *J. Phys. Chem. B* **2009**, *113*, 2177-2183.
36. Collins, L.V.; Kristian, S.; Weidenmaier, C.; Faigle, M.; Van Kessel, K.P.; Van Strijp, J.A.; Götz, F.; Neumeister, B.; Peschel, A. *J. Infect. Dis.* **2002**, *186*, 214-219.
37. Peschel, A.; Otto, M.; Jack, R.W.; Kalbacher, H.; Jung, G.; Götz, F. *J. Biol. Chem.* **1999**, *274*, 8405-8410.
38. Kristian, S.; Lauth, X.; Nizet, V.; Götz, F.; Neumeister, B.; Peschel, A.; Landmann, R. *J. Infect. Dis.* **2003**, *188*, 414-423.
39. Peschel, A.; Sahl, H.-G. *Nat. Rev. Microbiol.* **2006**, *4*, 529-536.
40. Sieradzki, K.; Tomasz, A. *J. Bacteriol.* **2003**, *185*, 7103-7110.
41. Bae, T.; Banger, A.K.; Wallace, A.; Glass, E.M.; Aslund, F.; Schneewind, O.; Missiakas, D.M. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12312-12317.
42. Faure, E.; Equils, O.; Sieling, P.A.; Thomas, L.; Zhang, F.X.; Kirschning, C.J.; Polentarutti, N.; Muzio, M.; Arditi, M. *J. Biol. Chem.* **2000**, *275*, 11058-11063.
43. Schmidt, R.R.; Pedersen, C.M.; Qiao, Y.; Zähringer, U. *Org. Biomol. Chem.* **2011**, *9*, 2040-2052.
44. Morath, S.; Von Aulock, S.; Hartung, T. *J. Endotoxin Res.* **2005**, *11*, 348-356.
45. Dessing, M.C.; Schouten, M.; Draing, C.; Levi, M.; Von Aulock, S.; Van der Poll, T. *J. Infect. Dis.* **2008**, *197*, 245-252.
46. Morath, S.; Stadelmaier, A.; Geyer, A.; Schmidt, R.R.; Hartung, T. *J. Exp. Med.* **2002**, *195*, 1635-1640.
47. Hashimoto, M.; Tawaratsumida, K.; Kariya, H.; Kiyohara, A.; Suda, Y.; Kirikae, F.; Kirikae, T.; Götz, F. *J. Immunol.* **2006**, *177*, 3162-3169.
48. Kang, J.K.; Nan, X.; Jin, M.S.; Youn, S.-J.; Ryu, Y.H.; Mah, S.; Han, S.H.; Lee, H.; Paik, S.-G.; Lee, J.-O. *Immunity* **2009**, *31*, 873-884.
49. Hummell, D.S.; Swift, A.J.; Tomasz, A.; Winkelstein, J.A. *Infect. Immun.* **1985**, *47*, 384-387.
50. Walsh, S.; Kokai-Kun, J.; Shah, A.; Mond, J. *Pharm. Res.* **2004**, *21*, 1770-1775.
51. Kumar, A.; Ray, P.; Kanwar, M.; Sharma, M.; Varma, S. *Int. J. Med. Sci.* **2005**, *2*, 129-136.
52. Theilacker, C.; Krueger, W.A.; Kropec, A.; Huebner, J. *Vaccine*, **2004**, *22S*, 31-38.
53. Robbins, J.B.; Schneerson, R.; Szu, S.C. *J. Infect. Dis.* **1995**, *171*, 1387-1398.
54. Vliegenthart, J.F.G. *FEBS Lett.* **2006**, *580*, 2945-2950.
55. Bundle, D.R. *Nat. Chem. Biol.* **2007**, *3*, 605-606.
56. Hecht, M.-L.; Stallforth, P.; Varón Silva, D.; Adibekian, A.; Seeberger, P.H. *Curr. Opin. Chem. Biol.* **2009**, *13*, 354-359.
57. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Sava, I.; Ye, L.; Bychowska, A.; Holst, O.; Huebner, J. *Plos One* **2011**, *6*, e17839.
58. Wang, Y.; Huebner, J.; Tzianabos, A.O.; Martirosian, G.; Kasper, D.L.; Pier, G.B. *Carbohydr. Res.* **1999**, *316*, 155-160.
59. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Fabretti, F.; Sange, T.; Holst, O.; Huebner, J. *Infect. Immun.* **2006**, *74*, 5703-5712.
60. Theilacker, C.; Kropec, A.; Hammer, F.; Sava, I.; Wobser, D.; Sakinc, T.; Codée, J.D.C.; Hogendorf, W.F.J.; Van der Marel, G.A.; Huebner, J. *J. Infect. Dis.* **2012**, *205*, 1076-1085.
61. Reese, C.B. *Org. Biomol. Chem.* **2005**, *3*, 3851-3868.
62. Michelson, A.M.; Todd, A.R. *J. Chem. Soc.* **1955**, 2632-2638.
63. Khorana, H.G.; Tener, G.M.; Moffatt, J.G.; Pol, E.H. *Chem. Ind.* **1956**, 1523.
64. Khorana, H.G.; Razzell, W.E.; Gilham, P.T.; Tener, G.M.; Pol, E.H. *J. Am. Chem. Soc.* **1957**, *79*, 1002-1003.
65. Gilham, P.T.; Khorana, H.G. *J. Am. Chem. Soc.* **1958**, *80*, 6212-6222.

66. Agarwal, K.L.; Yamazaki, A.; Cashion, P.J.; Khorana, H.G. *Angew. Chem., Int. Ed.* **1972**, *11*, 451-459.
67. Khorana, H.G. *Pure Appl. Chem.* **1968**, *17*, 349-381.
68. Letsinger, R.L.; Mahadevan, V. *J. Am. Chem. Soc.* **1965**, *87*, 3526-3527.
69. Letsinger, R.L.; Ogilvie, K.K. *J. Am. Chem. Soc.* **1967**, *89*, 4801-4803.
70. Reese, C.B.; Saffhill, R. *Chem. Commun.* **1968**, 767-768.
71. Eckstein, F.; Rizk, I. *Angew. Chem., Int. Ed.* **1967**, *6*, 695-696.
72. Van Boom, J.H.; Burgers, P.M.J.; Van Deursen, P.H.; Arentzen, R.; Reese, C.B. *Tetrahedron Lett.* **1974**, *15*, 3785-3788.
73. Van der Marel, G.A.; Van Boeckel, C.A.A.; Wille, G.; Van Boom, J.H. *Tetrahedron Lett.* **1981**, *22*, 3887-3890.
74. Hall, R.H.; Todd, A.R.; Webb, R.F. *J. Chem. Soc.* **1957**, 3291-3296.
75. Garegg, P.J.; Regberg, T.; Stawinski, J.; Strömberg, R. *Chem. Scr.* **1985**, *25*, 280-282.
76. Garegg, P.J.; Lindl, I.; Regberg, T.; Stawinski, J.; Strömberg, R. *Tetrahedron Lett.* **1986**, *27*, 4051-4054.
77. Froehler, B.C.; Matteuci, M.D. *Tetrahedron Lett.* **1986**, *27*, 469-472.
78. Froehler, B.C.; Ng, P.G.; Matteuci, M.D. *Nucleic Acids Res.* **1986**, *14*, 5399-5407.
79. Westheimer, F.H.; Huang, S.; Covitz, F. *J. Am. Chem. Soc.* **1988**, *110*, 181-185.
80. Dreef, C.E.; Dreef-Tromp, C.M.; Van der Marel, G.A.; Van Boom, J.H. *Synlett.* **1990**, 481-483.
81. Beaucage, S.L.; Caruthers, M.H. *Tetrahedron Lett.* **1981**, *22*, 1859-1862.
82. Letsinger, R.L.; Lunsford, W.B. *J. Am. Chem. Soc.* **1976**, *98*, 3655-3661.
83. Adams, S.P.; Kavka, K.S.; Wykes, E.J.; Holder, S.B.; Gallupi, G.R. *J. Am. Chem. Soc.* **1983**, *105*, 661-663.
84. McBride, L.J.; Caruthers, M.H. *Tetrahedron Lett.* **1983**, *24*, 245-248.
85. Sinha, N.D.; Biernat, J.; Köster, H. *Tetrahedron Lett.* **1983**, *24*, 5843-5846.
86. Beaucage, S.L.; Iyer, R.P. *Tetrahedron* **1992**, *48*, 2223-2311.
87. Crisel, R.M.; Baker, R.S.; Dorman, D.E. *J. Biol. Chem.* **1975**, *250*, 4926-4930.
88. Garegg, P.J.; Lindberg, B.; Samuelsson, B. *Carbohydr. Res.* **1977**, *58*, 219-221.
89. Hoogerhout, P.; Evenber, D.; Van Boeckel, C.A.A.; Poolman, J.T.; Beuvery, E.C.; Van der Marel, G.A.; Van Boom, J.H. *Tetrahedron Lett.* **1987**, *28*, 1553-1556.
90. Hoogerhout, P.; Funke, C.W.; Mellema, J.R.; Van Boeckel, C.A.A.; Evenberg, D.; Poolman, J.T.; Lefeber, A.W.M.; Van der Marel, G.A.; Van Boom, J.H. *J. Carbohydr. Chem.* **1988**, *7*, 399-416.
91. Nilsson, S.; Bengtsson, M.; Norberg, T. *J. Carbohydr. Chem.* **1992**, *11*, 265-285.
92. Elie, C.J.J.; Muntendam, H.J.; Van den Elst, H.; Van der Marel, G.A.; Van Boom, J.H.; Hoogerhout, P. *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 219-223.
93. Kandil, A.A.; Chan, N.; Chong, P.; Klein, M. *Synlett* **1992**, *7*, 555-557.
94. Verez-Bencomo, V.; Fernández-Santana, V.; Hardy, E.; Toledo, M.E.; Rodríguez, M.C.; Heynngnezz, L.; Rodriguez, A.; Baly, A.; Herrera, L.; Izquierdo, M.; Villar, A.; Valdés, Y.; Cosme, K.; Deler, M.L.; Montane, M.; Garcia, E.; Ramos, A.; Aguilar, A.; Medina, E.; Toraño, G.; Sosa, I.; Hernandez, I.; Martínez, R.; Muzachio, A.; Carmenates, A.; Costa, L.; Cardoso, F.; Campa, C.; Diaz, M.; Roy, R. *Science* **2004**, *305*, 522-525.
95. Van Boeckel, C.A.A.; Visser, G.M.; Hermans, J.P.G.; Van Boom, J.H. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 526-537.
96. Ohtsuka, E.; Murao, K.; Ubasawa, M.; Ikehara, M. *J. Am. Chem. Soc.* **1970**, *92*, 3441-3445.
97. Ikehara, M.; Uesugi, S.; Fukui, T. *Chem. Pharm. Bull.* **1967**, *15*, 440-447.
98. Oltvoort, J.J.; Van Boeckel, C.A.A.; De Koning, J.H.; Van Boom, J.H. *Recl. Trav. Chim. Pays-Bas* **1982**, *101*, 87-91.



99. Oltvoort, J.J.; Kloosterman, M.; Van Boeckel, C.A.A.; Van Boom, J.H. *Carbohydr. Res.* **1984**, *130*, 147-163.
100. Westerduin, P.; Veeneman, G.H.; Pennings, Y.; Van der Marel, G.A.; Van Boom, J.H. *Tetrahedron Lett.* **1987**, *28*, 1557-1560.
101. Fukase, K.; Matsumoto, T.; Ito, N.; Yoshimura, T.; Kotani, S.; Kusumoto, S. *Bull. Chem. Soc.* **1992**, *65*, 2643-2654.
102. Fukase, K.; Yoshimura, T.; Kotani, S.; Kusumoto, S. *Bull. Chem. Soc.* **1994**, *67*, 473-482.
103. Takada, H.; Kawabata, Y.; Arakaki, R.; Kusumoto, S.; Fukase, K.; Suda, Y.; Yoshimura, T.; Koeguchi, S.; Kato, K.; Komuro, T. *Infect. Immun.* **1995**, *63*, 57-65.
104. Fekete, A.; Hoogerhout, P.; Zomer, G.; Kubler-Kielb, J.; Schneerson, R.; Robbins, J.B.; Pozsgay, V. *Carbohydr. Res.* **2006**, *341*, 2037-2048.
105. Kubler-Kielb, J.; Pozsgay, V. *J. Org. Chem.* **2005**, *70*, 6987-6990.
106. Stadelmaier, A.; Morath, S.; Hartung, T.; Schmidt, R.R. *Angew. Chem. Int. Ed.* **2003**, *42*, 916-920.
107. Figueroa-Perez, I.; Stadelmaier, A.; Morath, S.; Hartung, T.; Schmidt, R.R. *Tetrahedron: Asymm.* **2005**, *16*, 493-506.
108. Deininger, S.; Figueroa-Perez, I.; Sigel, S.; Stadelmaier, A.; Schmidt, R.R.; Hartung, T.; Von Aulock, S. *Clin. Vaccine Immunol.* **2007**, *14*, 1629-1633.
109. Figueroa-Perez, I.; Stadelmaier, A.; Deininger, S.; Von Aulock, S.; Hartung, T.; Schmidt, R.R. *Carbohydr. Res.* **2006**, *341*, 2901-2911.
110. Stadelmaier, A.; Figueroa-Perez, I.; Deininger, S.; von Aulock, S.; Hartung, T.; Schmidt, R.R. *Bioorg. Med. Chem.* **2006**, *14*, 6239-6254.
111. Roethlisberger, P.; Lida-Tanaka, N.; Hollemeyer, K.; Heinze, E.; Ishizuka, I.; Fischer, W. *Eur. J. Biochem.* **2000**, *267*, 5520-5530.
112. Qiao, Y.; Lindner, B.; Zähringer, U.; Truog, P.; Schmidt, R.R. *Bioorg. Med. Chem.* **2010**, *18*, 3696-3702.
113. Draing, C.; Pfitzenmaier, M.; Zummo, S.; Mancuso, G.; Geyer, A.; Hartung, T.; von Aulock, S. *J. Biol. Chem.* **2006**, *281*, 33849-33859.
114. Pedersen, C. M.; Figueroa-Perez, I.; Lindner, B.; Ulmer, A. J.; Zähringer, U.; Schmidt, R. R. *Angew. Chem.* **2010**, *122*, 2639-2644; *Angew. Chem., Int. Ed.* **2010**, *49*, 2585-2590.
115. Pedersen, C. M.; Figueroa-Perez, I.; Boruwa, J.; Lindner, B.; Ulmer, A. J.; Zähringer, U.; Schmidt, R. R. *Chem. Eur. J.* **2010**, *16*, 12627-12641.
116. Pedersen, C. M.; Figueroa-Perez, I.; Ulmer, A. J.; Zähringer, U.; Schmidt, R. R. *Tetrahedron* **2012**, *68*, 1052-1061.

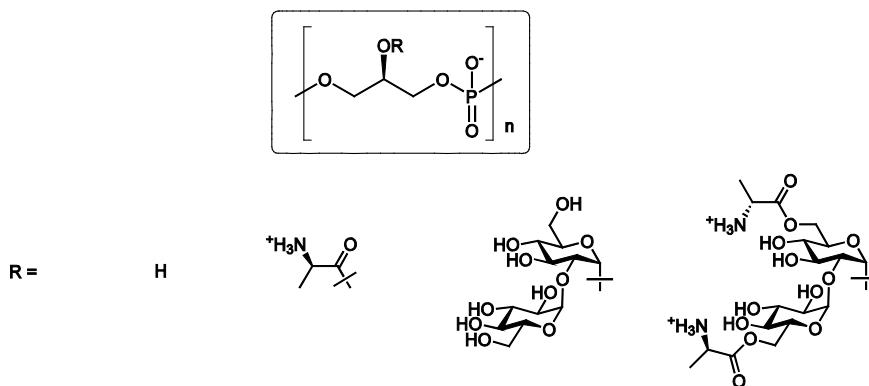
## Chapter 2

# Synthesis of an $\alpha$ -kajibiosyl GTA Hexamer

### Introduction

Antibiotic resistance is an emerging problem in society and prevention strategies, such as vaccination with (synthetic) cell-wall antigens, have become a promising approach. As described in **chapter 1** teichoic acids (TAs) are Gram-positive cell wall components that modulate the mammalian immune system. In order to understand the molecular mode of action of these molecules, well defined fragments could be a valuable tool.<sup>1-5</sup> Synthetic studies to and biological evaluation of *Staphylococcus aureus* LTA fragments by Schmidt and Hartung led to more insight in the minimal structural requirements for cytokine induction.<sup>6-12</sup>

*Enterococcus faecalis* is a commensal bacterium and generally of low virulence. However, with the emergence of vancomycin-resistant strains an increasing amount of *E. faecalis* infections is reported.<sup>13-15</sup> The TA of *E. faecalis* is antigenic and built up from 1,3-poly(glycerolphosphate), randomly decorated at the C-2 positions with D-alanine, kojibiose ( $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -D-glucose), and 6,6'-di-alanyl- $\alpha$ -kajibiose, as depicted in Figure 1.<sup>16,17</sup>



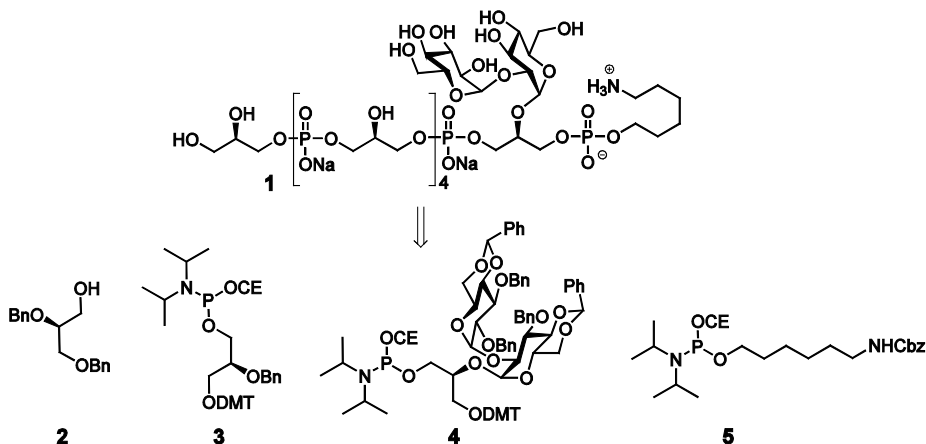
**Figure 1.** General structure of *E. faecalis* GTA and its substituents.

This chapter deals with the synthesis of a glycerol TA hexamer, containing an  $\alpha$ -kajibiosyl substitution.<sup>18</sup> The syntheses of the stereochemically pure (kajibiosyl) glycerol synthons and their use in the construction of a hexamer (kajibiosyl)glycerol phosphate is described. Installation of the phosphodiester bridges was performed

using phosphoramidite chemistry, which has emerged in the past two decades as the method of choice for (automated) oligonucleotide synthesis.<sup>19-21</sup>

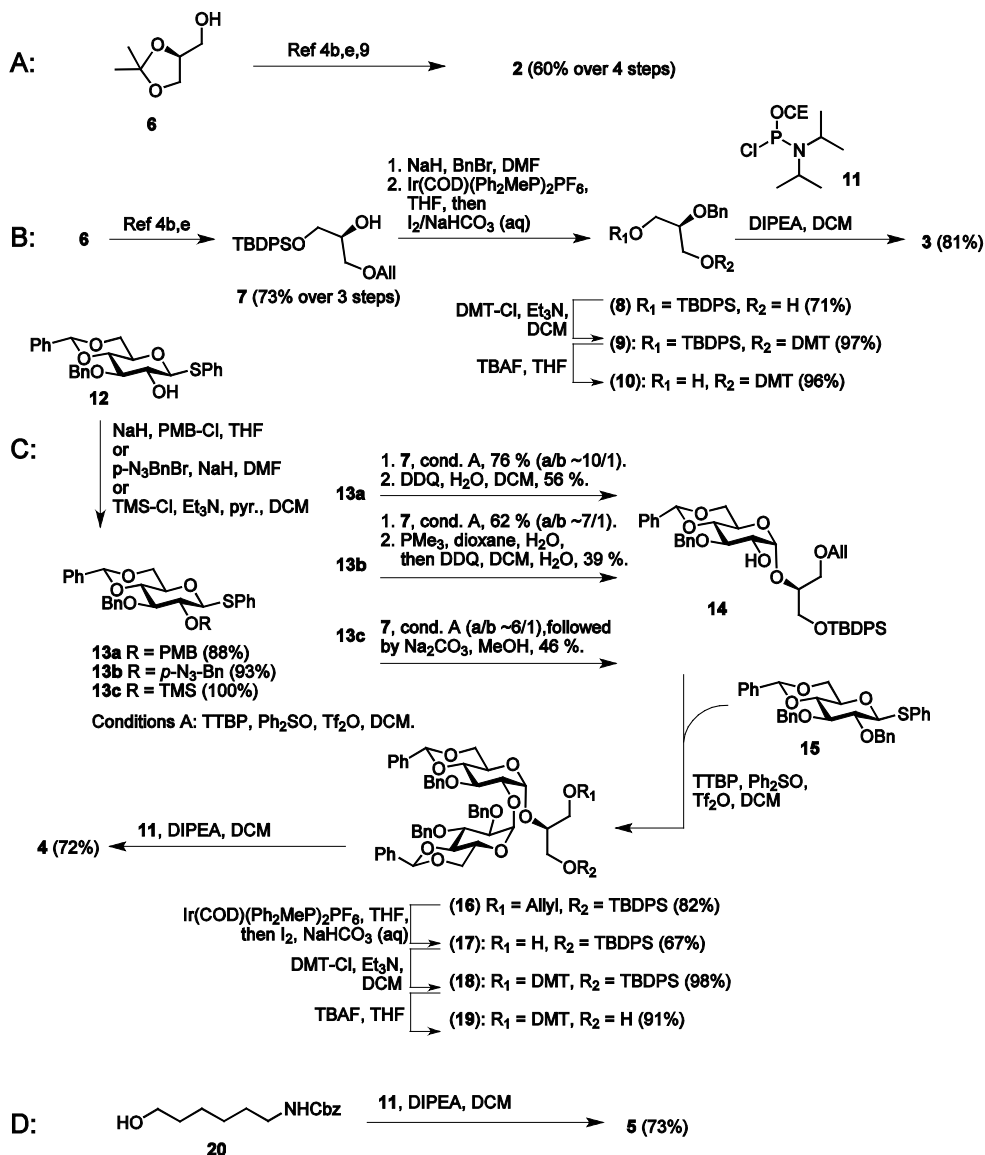
## Results and Discussion

**Scheme 1.** Target hexamer **1** and the required phosphoramidites **2-5**.



As retrosynthetically depicted in Scheme 1, TA hexamer **1** can be assembled from four synthons: dibenzyl glycerol **2**, the starting building block; glycerol phosphoramidite **3** and kojibiosyl glycerol phosphoramidite **4**, used for chain elongation, and aminohexylphosphoramidite **5**, the terminal building block. The primary amino group of the hexyl spacer presents a chemoselective ligation handle for attachment of, for instance, carrier proteins or fluorescent tags (see **chapter 7**). The 2-cyanoethyl (CE) was chosen as a phosphate protecting group because of its robustness during the synthesis and ease of removal at the end of the synthesis. The hydroxyl functions and the amino group of the aminohexyl spacer were masked with benzyl type protecting groups, which facilitated global deprotection in the final stage of the hexamer assembly by hydrogenolysis. As a temporary protecting group the dimethoxytrityl (DMT) group has been selected because it has proven its merits in contemporary DNA and RNA synthesis protocols and can be cleaved under mildly acidic conditions.<sup>21,22</sup>

The synthesis of the four building blocks is depicted in Scheme 2. Dibenzylglycerol **2** was synthesized according to literature procedures from solketal **6** (Scheme 2A).<sup>7,10,23,24</sup> Solketal **6** also served as starting compound in the synthesis of glycerolphosphoramidite **3**. Allylation of the free hydroxyl function, acidic hydrolysis of the isopropylidene and subsequent selective silylation of the primary alcohol yielded partially protected glycerol **7** (Scheme 2B).<sup>7</sup> The remaining hydroxyl in compound **7** was benzylated to provide the fully protected glycerol in high yield, containing a minor amount (~3%) of 1-*O*-benzyl-2-*O*-*tert*-butyldiphenylsilyl-3-*O*-allyl-

**Scheme 2.** Synthesis of (phosphoramidite) building-blocks 2-5.


*sn*-glycerol, which resulted from silyl migration during the basic etherification, and which could not be removed by silica gel chromatography. To liberate the C3 hydroxyl, the allyl ether was isomerized using Ir(COD)(Ph<sub>2</sub>MeP)<sub>2</sub>PF<sub>6</sub>.<sup>25</sup> Subsequent enol ether cleavage using iodine and aqueous NaHCO<sub>3</sub> afforded glycerol **8** in 71% over 2 steps. Dimethoxytritylation of the primary alcohol furnished glycerol **9**, which was desilylated to provide alcohol **10**. At this stage the product could be separated by silica gel chromatography from its regioisomeric impurity. Finally, installment of the

phosphoramidite moiety using 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite **11** provided building block **3**.

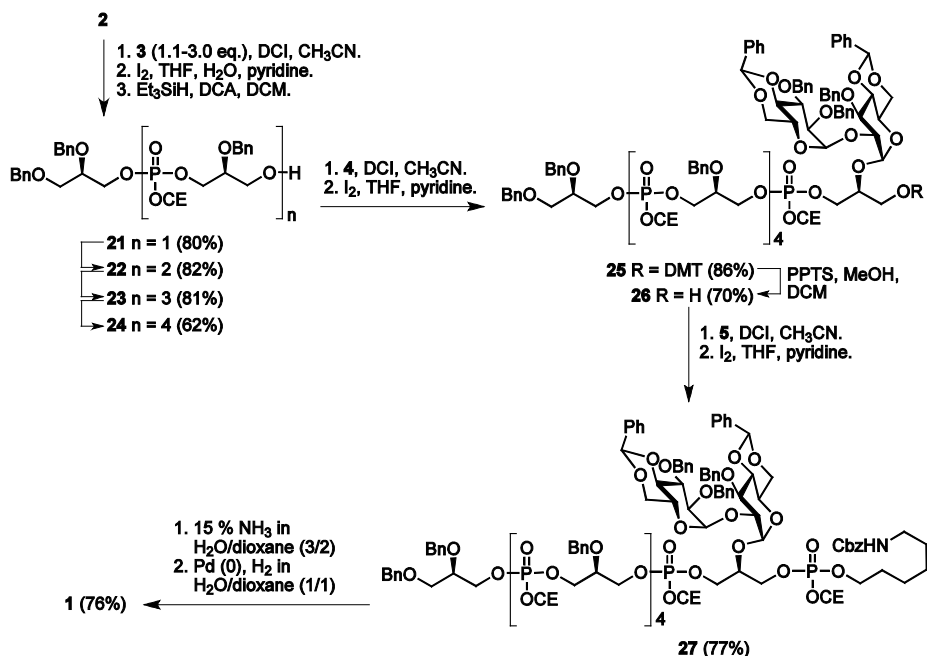
With the first two building blocks in hand the synthesis of  $\alpha$ -kajibiosylglycerol synthon **4**, containing two 1,2-*cis* glucosidic linkages, was undertaken. Although tremendous progress has been made in the stereoselective construction of glycosidic bonds, the installment of two 1,2-*cis*-glucosyl linkages still presents a significant challenge. Recently, Boons and co-workers revealed an elegant approach to this long standing problem, by installing  $\alpha$ -directing ethyl (S)-mandelate or (1S)-phenyl(thiophenyl)ethyl ethers on the 2' hydroxyl of glucose donors.<sup>26-28</sup> Unfortunately, the assembly of the kojibiose glycerol pseudotrisaccharide requires the glycosylation of the first glucose residue at the C-2 hydroxyl and the latter ether protecting groups cannot be removed selectively while keeping the other benzyl ethers intact. Alternative methods which have been used to construct 1,2-*cis* glucosidic linkages include Lemieux's *in situ* anomerization protocol<sup>29,30</sup> and the employment of large substituents such as the DMT group at the glucosyl C-6 hydroxyl.<sup>31-33</sup> Given the relatively low reactivity of the secondary alcohol function which has to be glucosylated the former approach was deemed unsuitable. The latter approach was not pursued at this point because this would exclude the use of trityl-type groups in the glycerol part of the pseudotrisaccharide, which was a prerequisite in the design of our protecting group strategy (*vide supra*).<sup>34</sup> An alternative approach to the formation of 1,2-*cis* glucosidic linkages has been described by Crich and co-workers, using benzylidene protected thioglucosyl donors.<sup>35</sup> Three 4,6-*O*-benzylidene functionalized glucosyl donors, having either a C2 *para*-methoxybenzyl (PMB, **13a**), a C2 *para*-azidobenzyl (PAB, **13b**) or a C2 trimethylsilyl (TMS, **13c**) protecting group, were explored (Scheme 2C). The installment of the *para*-methoxybenzyl group in construct **12**<sup>36</sup> led to benzylidene glucoside **13a** in 88%. This thioglycoside was then condensed with glycerol acceptor **7** using the Ph<sub>2</sub>SO/Tf<sub>2</sub>O activator system<sup>37</sup> to provide the intermediate pseudodisaccharide in good yield and excellent selectivity ( $\alpha/\beta$  = 10/1). Subsequent treatment of the fully protected adduct with DDQ gave alcohol **14** in moderate yield (56%), resulting in a 37% total yield of  $\alpha$ -glucosyl glycerol **14** from alcohol **12**. A similar series of reactions was employed to prepare pseudodisaccharide **14** with the use of the *para*-azido benzyl ether<sup>38</sup> instead of the *para*-methoxy benzyl group as a non-participating group at C-2. In this case (**13b**), the benzylation reaction proceeded uneventfully and the ensuing glycosylation reaction provided the *cis*-coupled product in good selectivity (62%,  $\alpha/\beta$  = 7/1). Unfortunately, liberation of the C2 hydroxyl via a reduction-oxidation sequence provided target alcohol **14** in only 39%, to complete the four step sequence in a total yield of 22% of **14** from building block **12**. The last non-participating group that was tested was the trimethylsilyl ether (TMS). Donor **13c** was obtained quantitatively from **12**, and used in the subsequent glycosylation reaction without purification. The coupling of TMS protected thioglucose **13c** and glycerol **7** was immediately followed by treatment of the resulting pseudodisaccharide (~6:1 mixture of  $\alpha$  and  $\beta$ -anomers) with sodium

carbonate in methanol to yield **14** in 46% over the three steps, using a single chromatographical purification step, making this the most efficient route.

With the first 1,2-*cis* glycosidic linkage in place, the synthesis was continued with the condensation of dibenzyl benzylidene thioglucoside **15** and alcohol **14**. Gratifyingly, the pseudotrisaccharide (**16**) was obtained as a single diastereoisomer in 82% yield. This trisaccharide was transformed into the phosphoramidite building block **4** following the same sequence of reactions as described for the transformation of 3-*O*-allyl-2-*O*-benzyl-1-*O*-TBDPS-*sn*-glycerol into phosphoramidite **3**. Briefly, the allyl ether in **16** was removed by iridium catalyzed isomerisation and iodine mediated cleavage of the intermediate enol ether. The DMT group was installed, after which the TBDPS ether was removed by fluoride treatment and the primary alcohol was phosphitylated to complete the synthesis of the kojibiosyl glycerol **4**. To complete the set of target synthons, benzyloxycarbonyl protected aminohexanol **20** was treated with DiPEA and chlorophosphoramidite **11** to give phosphoramidite **5** (Scheme 2D).

With all necessary building blocks in hand the assembly of the hexamer was undertaken (Scheme 3). In the first step dibenzylglycerol **2** and glycerol phosphoramidite **3** were coupled with the use of 4,5-dicyanoimidazole (DCI) in acetonitrile. After oxidation of the intermediate phosphite (**12** in THF/pyridine 4/1), the crude intermediate was detritylated using dichloroacetic acid and triethylsilane in

**Scheme 3.** Synthesis of kojibiosyl GTA hexamer **1**.



dry DCM, giving dimer **21** in 80% yield. Repetition of this reaction sequence led to the consecutive formation of trimer **22** in 82% from **21**, tetramer **23** in 81% from **22** and pentamer **24** in 62% from **23**. Next, kojibiosylglycerol phosphoramidite **4** was coupled to the glycerolphosphate chain and, after iodine mediated oxidation, hexamer **25** was obtained in 86% yield. Apparently, the relatively bulky kojibiosyl substituent did not have an adverse effect on the formation of the mixed phosphotriester. To avoid unwanted acidolysis of the benzylidene functionalities in **25** the mild pyridinium *para*-toluenesulphonate (PPTS)/MeOH cocktail was employed for the cleavage of the DMT group.<sup>39</sup> Alcohol **26** was obtained in 70% yield when 1 mg/ml PPTS in MeOH/DCM (9/1) was employed. Subsequent coupling of spacer phosphoramidite **5** and oxidation gave the fully protected target compound **27** in 77% yield.

The deprotection sequence started with ammonolysis of the cyanoethyl groups in **27**, giving the hexameric phosphodiester quantitatively. Finally, all benzyl ethers, the two benzylidene acetals and the benzyloxycarbamate were removed using H<sub>2</sub>/Pd(0) in water/dioxane/AcOH. After size-exclusion chromatography, repeated lyophilization, and ion exchange the target *E. faecalis* TA-hexamer (**1**) was obtained as the per sodium salt in 76% yield. Hexamer **1** was evaluated in an opsonophagocytic killing inhibition assay (OPIA), to determine its binding to antibodies raised against native (*Enterococcal*) LTA, showing some inhibitory activity (compound **22**, figure 1, chapter three).

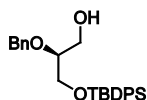
## Conclusion

In summary, this chapter describes the synthesis of a kojibiose containing *Enterococcus faecalis* teichoic acid hexamer (**1**). The teichoic acid fragment was constructed by coupling of suitably protected glycerol and  $\alpha$ -kojibiosyl substituted glycerol phosphoramidites. The key kojibiosyl synthon was obtained via a sequence of reactions involving two successive stereoselective  $\alpha$ -glucosylations. The use of a benzylidene thioglucose donor, temporarily protected at the C2-hydroxyl with a TMS-group proved to be the most efficient in the synthesis of the kojibiosyl glycerol pseudotrisaccharide. The phosphoramidite chemistry used for the assembly of the teichoic acid hexamer is compatible with an automated solid-phase approach, as is illustrated in chapter 3.<sup>40</sup>

## Experimental section

**General Procedures and Material:** All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma-Aldrich, Genscript) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Tf<sub>2</sub>O was distilled over P<sub>2</sub>O<sub>5</sub> using flame-dried glass-ware. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H<sub>2</sub>SO<sub>4</sub> in EtOH or with a solution of (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>·4H<sub>2</sub>O 25 g/l and (NH<sub>4</sub>)<sub>4</sub>Ce(SO<sub>4</sub>)<sub>4</sub>·2H<sub>2</sub>O 10 g/l, in 10% aqueous H<sub>2</sub>SO<sub>4</sub> followed by charring at +/- 140 °C. Some

unsaturated compounds were visualized by spraying with a solution of  $\text{KMnO}_4$  (2%) and  $\text{K}_2\text{CO}_3$  (1%) in  $\text{H}_2\text{O}$ . Optical rotation measurements ( $[\alpha]_{\text{D}}^{20}$ ) were performed on a Propol automated polarimeter (Sodium D-line,  $\lambda = 589 \text{ nm}$ ) with a concentration of 10 mg/ml ( $c = 1$ ), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300 and are reported in  $\text{cm}^{-1}$ .  $^{31}\text{P}$ ,  $^1\text{H}$ , and  $^{13}\text{C}$  NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 100 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in  $\text{CDCl}_3$  with chemical shift ( $\delta$ ) relative to tetramethylsilane, unless stated otherwise. High resolution mass spectra were recorded by direct injection (2  $\mu\text{l}$  of a 2  $\mu\text{M}$  solution in water/acetonitrile; 50/50; v/v and 0.1 % formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250  $^\circ\text{C}$ ) with resolution  $R = 60000$  at  $m/z$  400 (mass range  $m/z = 150\text{--}2000$ ) and dioctylphthalate ( $m/z = 391.28428$ ) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). TLC-MS (ESI) spectra of phosphoramidites **3**, **4** and **5** were obtained by analysis of TLC-plates (Schleicher & Schuell, treated with  $\text{Et}_3\text{N}$  before applying the phosphoramidites) with a CAMAG TLC-MS interface coupled to a Perkin Elmer Sciex API 165 mass instrument.

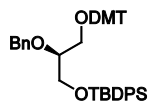


#### 1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-benzyl-*sn*-glycerol (**8**)

Silyl ether **7** (22.8 g, 61.5 mmol) was dissolved in DMF (200 ml) and cooled to 0  $^\circ\text{C}$ . After the addition of benzyl bromide (11.1 ml, 92.5 mmol) and heptane flushed NaH (60 % dispersion in mineral oil, 3.70 g, 92.5 mmol), the mixture was allowed to stir for 1.5 h.  $\text{H}_2\text{O}$  (50 ml) was added, and after addition of  $\text{Et}_2\text{O}$  (1.0 l), the mixture was washed with  $\text{H}_2\text{O}$  (2 x 200 ml) and brine (200 ml). The organic layer was dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. Repeated co-evaporation of the residue with  $\text{H}_2\text{O}$  (5 x 100 ml) and toluene (5 x 100 ml), followed by column chromatography (toluene/PE) resulted in the benzylated adduct (28.3 g, 61.4 mmol, 100%, containing ~3% 1-*O*-benzyl-2-*O*-*tert*-butyldiphenylsilyl-3-*O*-allyl-*sn*-glycerol) as a colourless oil. (analytical data:  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): -10.4; IR: 737, 924, 1103, 1427, 2856  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz):  $\delta = 1.05$  (s, 9H, *t*-Bu TBDPS), 3.55 - 3.71 (m, 3H, CH glycerol, 2 x CHH glycerol), 3.77 - 3.80 (m, 2H, 2 x CHH glycerol), 3.99 (m, 2H,  $\text{CH}_2$  allyl), 4.64 (s, 2H,  $\text{CH}_2$  Bn), 5.16 (m, 1H, CHH allyl), 5.26 (dd, 1H, 1,7 Hz, 17.3 Hz, CHH allyl), 5.86 - 5.93 (m, 1H, CH allyl), 7.18 - 7.43 (m, 11H,  $\text{H}_{\text{arom}}$ ), 7.66 - 7.71 (m, 4H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 19.2$  ( $\text{C}_q$  *t*-Bu), 26.8 (3 x  $\text{CH}_3$  TBDPS), 63.5 ( $\text{CH}_2$  glycerol), 70.2 ( $\text{CH}_2$  glycerol), 72.2, 72.3 ( $\text{CH}_2$  allyl,  $\text{CH}_2$  Bn), 78.8 (CH glycerol), 116.8 ( $\text{CH}_2$  allyl), 127.4, 127.7, 128.2, 129.6 ( $\text{CH}_{\text{arom}}$ ), 133.5 (2 x  $\text{C}_q$  phenyl), 134.8 (CH allyl), 135.6 ( $\text{CH}_{\text{arom}}$ ), 138.8 ( $\text{C}_q$  Bn).) A solution of the intermediate (9.21 g, 20.0 mmol) in freshly distilled THF (125 ml) was stirred under argon atmosphere for 30 min. After addition of  $\text{Ir}(\text{COD})(\text{Ph}_2\text{MeP})_2\text{PF}_6$  (423 mg, 0.50 mmol, 2.5 mol %), the solution turned red. The solution was then purged with  $\text{H}_2$  (g) for ~30s, after which it was stirred for 2 h under argon atmosphere. The solution was diluted with THF (250 ml) and, after addition of sat. aq.  $\text{NaHCO}_3$  (300 ml) and  $\text{I}_2$  (7.61 g, 30.0 mmol), the mixture was allowed to stir overnight. The mixture was diluted with  $\text{EtOAc}$  (1.5 l) and washed with sat. aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (2 x 300 ml) and brine (200 ml). The organic layer was dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. Purification of the residue by column chromatography ( $\text{EtOAc/PE}$ ), yielded **8** (6.26 g, 14.9 mmol, 75%, containing ~3% 1-*O*-benzyl-2-*O*-*tert*-butyldiphenylsilyl-*sn*-glycerol) as slightly yellow oil.  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): -22.0; IR: 739, 824, 1113, 1427, 2361, 2858  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz):  $\delta = 1.06$  (s, 9H, *t*-Bu TBDPS), 2.05 (t, 1H,  $J = 6.2 \text{ Hz}$ , OH), 3.60 - 3.82 (m, 5H, CH glycerol, 2 x  $\text{CH}_2$  glycerol), 4.51 (d, 1H  $J = 11.7 \text{ Hz}$ , CHH Bn), 4.63 (d, 1H,  $J = 11.7 \text{ Hz}$ , CHH Bn), 7.25 - 7.45 (m, 11H,  $\text{H}_{\text{arom}}$ ), 7.66 - 7.68 (m, 4H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 19.2$  ( $\text{C}_q$  *t*-Bu), 26.8 (3 x  $\text{CH}_3$  TBDPS), 62.8 ( $\text{CH}_2$  glycerol), 63.5 ( $\text{CH}_2$  glycerol), 72.1 ( $\text{CH}_2$  Bn), 79.6 (CH glycerol), 127.7, 128.4, 129.8

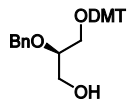


(CH<sub>arom</sub>), 133.1, 133.2 (2 x C<sub>q</sub> phenyl), 135.6 (CH<sub>arom</sub>), 138.3 (C<sub>q</sub> Bn); HRMS: C<sub>26</sub>H<sub>32</sub>O<sub>3</sub>Si + Na<sup>+</sup> requires 443.2013, found 443.2013.



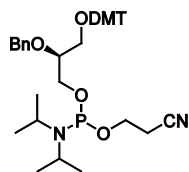
### 1-*O*-(*tert*-Butyldiphenylsilyl)-2-*O*-benzyl-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (**9**)

Alcohol **8** (6.18 g, 14.7 mmol) in DCM (100 ml) was cooled to 0 °C. Added were Et<sub>3</sub>N (3.05 ml, 22.0 mmol) and 4,4'-dimethoxytrityl chloride (DMT-Cl, 5.47 g, 16.2 mmol), respectively, and the mixture was allowed to stir overnight at room temperature. The reaction was quenched by the addition of H<sub>2</sub>O (3 ml), and stirred for 15 min. After washing with H<sub>2</sub>O (30 ml) and brine (30 ml), the organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The resulting oil was further purified by column chromatography (EtOAc/PE/Et<sub>3</sub>N), giving **9** (10.3 g, 14.2 mmol, 97%, containing ~3% 1-*O*-benzyl-2-*O*-*tert*-butyldiphenylsilyl-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol) as colourless oil. [α]<sub>D</sub><sup>20</sup> (MeOH): +2.0; IR: 827, 1036, 1113, 1250, 1508, 1609, 2359, 2930 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz): δ = 0.97 (s, 9H, *t*-Bu TBDPS), 3.23 - 3.31 (m, 2H, 2 x CHH glycerol), 3.71 - 3.78 (m, 9H, 2 x OMe, 2 x CHH glycerol, CH glycerol), 4.66 (2 x d, 2H, *J* = 12.2 Hz, CH<sub>2</sub> Bn), 6.74 - 6.83 (m, 4H, H<sub>arom</sub>), 7.00 - 7.45 (m, 20H, H<sub>arom</sub>), 7.60 - 7.62 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 19.1 (C<sub>q</sub> *t*-Bu), 26.8 (3 x CH<sub>3</sub> TBDPS), 55.1 (2 x OMe) 63.7 (CH<sub>2</sub> glycerol), 63.9 (CH<sub>2</sub> glycerol), 72.3 (CH<sub>2</sub> Bn), 79.3 (CH glycerol), 86.0 (C<sub>q</sub> DMT), 113.0, 113.6 (CH<sub>arom</sub>), 126.1 - 130.3 (CH<sub>arom</sub>), 133.4, 133.5 (2 x C<sub>q</sub> phenyl), 136.3 (CH<sub>arom</sub>), 136.4, 138.9, 145.1, 158.3 (C<sub>q</sub> Bn, 5 x C<sub>q</sub> DMT); HRMS: C<sub>47</sub>H<sub>50</sub>O<sub>5</sub>Si + Na<sup>+</sup> requires 745.3320, found 745.3322.



### 2-*O*-Benzyl-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (**10**)

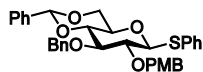
To a solution of glycerol derivative **9** (25.5 g, 35.3 mmol) in THF (200 ml), was added TBAF (1.0 M solution in THF, 53 ml). After stirring for 3 h at room temperature, the solvent was removed *in vacuo*. The residue was purified by column chromatography (EtOAc/PE/Et<sub>3</sub>N), affording primary alcohol **10** (16.4 g, 33.8 mmol, 96%) as colourless oil. [α]<sub>D</sub><sup>20</sup> (MeOH): +10.6; IR: 829, 1034, 1177, 1250, 1508, 1607, 2359 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz): δ = 2.02 (bs, 1H, OH), 3.21 - 3.31 (m, 2H, 2 x CHH glycerol), 3.63 - 3.67 (m, 2H, CH glycerol, CHH glycerol), 3.70 - 3.74 (m, 7H, 2 x OMe, CHH glycerol), 4.53 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.66 (d, 1H, *J* = 12.0 Hz, CHH Bn), 6.80 (ad, 4H, *J* = 8.8 Hz, H<sub>arom</sub>), 7.16 - 7.38 (m, 12H, H<sub>arom</sub>), 7.44 (d, 2H, *J* = 7.6, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 55.0 (2 x OMe), 63.0 (CH<sub>2</sub> glycerol), 63.3 (CH<sub>2</sub> glycerol), 72.0 (CH<sub>2</sub> Bn), 78.9 (CH glycerol), 86.4 (C<sub>q</sub> DMTr), 113.2 (CH<sub>arom</sub>), 126.7 - 129.9 (CH<sub>arom</sub>), 135.9, 138.2, 144.7, 158.4 (C<sub>q</sub> Bn, 5 x C<sub>q</sub> DMTr); HRMS: C<sub>31</sub>H<sub>32</sub>O<sub>5</sub> + Na<sup>+</sup> requires 507.3142, found 507.3135.



### 1-([*N,N*-Diisopropylamino]-2-cyanoethylphosphite)-2-*O*-benzyl-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (**3**)

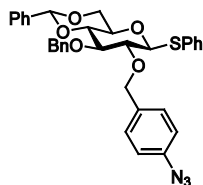
To a solution of **10** (3.19 g, 6.59 mmol) and DIPEA (1.72 ml, 9.88 mmol) in DCM (65 ml) were added activated MS3Å and (*N,N*-diisopropylamino)-2-cyanoethyl-chlorophosphite (**11**, 1.82 g, 7.90 mmol). After stirring for 2 h, the reaction was quenched with H<sub>2</sub>O (5.0 ml) and washed with H<sub>2</sub>O (50 ml) and brine (50 ml) respectively. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Column chromatography (EtOAc/heptane/Et<sub>3</sub>N) gave phosphoramidite **3** (3.74 g, 5.34 mmol, 81%, mixture of diastereoisomers) as colourless oil. <sup>31</sup>P NMR (161.7 MHz, CD<sub>3</sub>CN): δ = 149.2; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN): δ = 1.08 (t, 6H, *J* = 6.5 Hz, 2 x CH<sub>3</sub> isopropylamino), 1.14 (dd, 6H, *J* = 1.8 Hz, 6.8 Hz, 2 x CH<sub>3</sub> isopropylamino), 2.51 - 2.56 (m, 2H, CH<sub>2</sub> cyanoethyl), 3.17 - 3.24 (m, 2H, 2 x CH isopropylamino), 3.50 - 3.58 (m, 2H, 2 x CHH glycerol), 3.64 - 3.81 (m, 11H, CH glycerol, 2 x CHH glycerol, CH<sub>2</sub> cyanoethyl, 2 x OMe), 4.61 -

4.68 (m, 2H, CH<sub>2</sub> Bn), 6.83 (d, 4H,  $J$  = 8.9 Hz, H<sub>arom</sub>), 7.19 - 7.39 (m, 12H, H<sub>arom</sub>), 7.45 - 7.47 (m, 2H, H<sub>arom</sub>); TLC-MS: C<sub>40</sub>H<sub>49</sub>N<sub>2</sub>O<sub>6</sub>P + H<sup>+</sup> requires 685.34, found 685.5.



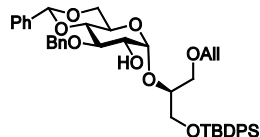
**Phenyl 2-O-(4-methoxybenzyl)-3-O-benzyl-4,6-O-benzylidene-1-thio- $\beta$ -D-glucopyranoside (13a)**

To a cooled (0 °C) solution of thioglucose **12** (18.3 g, 40.5 mmol) in THF (250 ml) were added, respectively, *p*-methoxybenzyl chloride (6.03 ml, 44.5 mmol) and NaH (60 % dispersion in mineral oil, 1.78 g, 44.5 mmol). After stirring for 5 h, the mixture was quenched with MeOH (5.0 ml), diluted with EtOAc (1.0 l) and washed with, sat. aq. NaHCO<sub>3</sub> (400 ml) and brine (400 ml). The organic layer was dried with MgSO<sub>4</sub> and concentrated *in vacuo*, after which crystallization (EtOAc/PE) yielded fully protected thioglucose derivative **13a** (20.4 g, 35.8 mmol, 88%) as white solid. M.p.: 146-147 °C; [ $\alpha$ ]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): -11.2; IR: 748, 818, 1030, 1088, 1250, 1516 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 3.45 (m, 1H, H-5), 3.50 (dd, 1H,  $J$  = 8.4 Hz, 9.7 Hz, H-2), 3.69 (at, 1H,  $J$  = 9.4 Hz, H4), 3.76 - 3.84 (m, 5H, OMe, H-3, H-6), 4.37 (dd, 1H,  $J$  = 5.0 Hz, 10.5 Hz, H-6), 4.73 - 4.81 (m, 4H, 3 x CHH Bn, H-1), 4.94 (d, 1H,  $J$  = 11.2 Hz, CHH Bn), 5.57 (s, 1H, CH benzylidene), 6.85 - 6.88 (m, 2H, H<sub>arom</sub>), 7.26 - 7.39 (m, 13H, H<sub>arom</sub>), 7.46 - 7.54 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 55.3 (OMe), 68.7 (C-6), 70.2 (C-5), 75.3, 75.5 (CH<sub>2</sub> Bn, CH<sub>2</sub> PMB), 80.1 (C-2), 81.4 (C-4), 83.0 (C-3), 88.3 (C-1), 101.1 (CH benzylidene), 113.8 (CH<sub>arom</sub>), 126.0 - 129.9 (CH<sub>arom</sub>), 130.2 (C<sub>q</sub> S-phenyl), 132.2 (CH<sub>arom</sub>), 133.2, 137.2, 138.3 (C<sub>q</sub> Bn, C<sub>q</sub> PMB, C<sub>q</sub> benzylidene), 159.4 (C<sub>q</sub> PMB); HRMS: C<sub>34</sub>H<sub>34</sub>O<sub>6</sub>S + H<sup>+</sup> requires 571.2149, found 571.2147.



**Phenyl 2-O-(4-azidobenzyl)-3-O-benzyl-4,6-O-benzylidene-1-thio- $\beta$ -D-glucopyranoside (13b)**

To a cooled (0 °C) solution of thioglucose **12** (895 mg, 1.99 mmol) in DMF (20 ml) were added *p*-azidobenzyl bromide (634 mg, 2.99 mmol) and NaH (60 % dispersion in mineral oil, 127 mg, 3.18 mmol) respectively. After stirring for 1.5 h, MeOH (2.0 ml) was added before the mixture was diluted with Et<sub>2</sub>O (80 ml) and washed with H<sub>2</sub>O (20 ml) and brine (20 ml). The organic layer was dried with MgSO<sub>4</sub> and concentrated *in vacuo*. The residue was taken up in EtOAc (5 ml) and crystallized upon addition of PE, yielding **13b** (1.07 g, 1.84 mmol, 93%) as ochreous solid. M.p.: 150-155 °C (decomp.); [ $\alpha$ ]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +3.0; IR: 991, 1088, 1508, 2112 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 3.44 - 3.51 (m, 2H, H-2, H-5), 3.70 (at, 1H,  $J$  = 9.4 Hz, H-4), 3.78 - 3.84 (m, 2H, H-3, H-6), 4.39 (dd, 1H,  $J$  = 5.2 Hz, 10.4 Hz, H-6), 4.73 - 4.82 (m, 4H, 3 x CHH Bn, H-1), 4.95 (d, 1H,  $J$  = 11.2 Hz, CHH Bn), 5.59 (s, 1H, CH benzylidene), 6.96 - 7.00 (m, 2H, H<sub>arom</sub>), 7.26 - 7.40 (m, 13H, H<sub>arom</sub>), 7.45 - 7.53 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 68.7 (C-6), 70.2 (C-5), 75.1, 75.3 (CH<sub>2</sub> Bn, CH<sub>2</sub> *p*-N<sub>3</sub>Bn), 80.3 (C-2), 81.5 (C-4), 82.9 (C-3), 88.2 (C-1), 101.1 (CH benzylidene), 118.9 (CH<sub>arom</sub>), 126.0 - 132.2 (CH<sub>arom</sub>), 133.0, 134.8, 137.2, 138.2, 139.5 (C<sub>q</sub> S-phenyl, C<sub>q</sub> Bn, 2 x C<sub>q</sub> *p*-N<sub>3</sub>Bn, C<sub>q</sub> benzylidene); HRMS: C<sub>34</sub>H<sub>31</sub>N<sub>3</sub>O<sub>5</sub>S + H<sup>+</sup> requires 582.2057, found 582.2056.



**1-O-(tert-Butyldiphenylsilyl)-2-O-(3-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranosyl)-3-O-allyl-sn-glycerol (14)**

From **13a**:

A solution of donor **13a** (2.85 g, 4.99 mmol), TTBP (2.86 g, 11.5 mmol) and Ph<sub>2</sub>SO (1.11 g, 5.50 mmol) in freshly distilled DCM (100 ml), together with activated MS3Å, was stirred under argon at RT for 30 min. Subsequently, the mixture was cooled to -75 °C and stirred for another 15 min. After the addition of Tf<sub>2</sub>O (0.93 ml, 5.5 mmol), the mixture was stirred for 45 min at -75 °C and,

subsequently, glycerol acceptor **7** (2.22 g, 5.99 mmol) in DCM (10 ml) was added. After stirring for 60 minutes at -75 °C, the mixture was allowed to, gradually, warm to room temperature (~3 h). The reaction was quenched upon addition of Et<sub>3</sub>N (2.0 ml, 14 mmol) and stirred for 30 min. After washing the mixture with sat. aq. NaHCO<sub>3</sub> (30 ml) and brine (30 ml), the organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The resulting oil was dissolved in pyridine (30 ml) and, after the addition of Ac<sub>2</sub>O (7 ml), stirred for 2 h. After removal of the solvents *in vacuo*, the residue was dissolved in EtOAc (200 ml) and washed with sat. aq. NaHCO<sub>3</sub> (2 x 60 ml) and brine (60 ml). The organic layer was dried over MgSO<sub>4</sub> and the solvent removed under reduced pressure. Purification of the resulting oil by column chromatography (EtOAc/PE) gave the fully protected product (3.16 g, 3.80 mmol, 76%,  $\alpha/\beta$  mixture, ~10/1), as colourless oil. IR: 737, 1088, 1369, 1454, 1751, 2855, 2924 cm<sup>-1</sup>; <sup>1</sup>H NMR  $\alpha$ -product (400 MHz):  $\delta$  = 1.05 (s, 9H, *t*-Bu), 3.51 - 3.65 (m, 4H, CH glycerol, CHH glycerol, H-2, H-6), 3.69 - 3.80 (m, 6H, OMe, 3 x CHH glycerol), 3.89 (m, 1H, H-5), 3.96 - 4.03 (m, 5H, CH<sub>2</sub> allyl, H-3, H-4, H-6), 4.66 (d, 1H, *J* = 11.7 Hz, CHH Bn), 4.71 (d, 1H, *J* = 11.7 Hz, CHH Bn), 4.79 (d, 1H, *J* = 11.2 Hz, CHH Bn), 4.85 (d, 1H, *J* = 11.3 Hz, CHH Bn), 5.17 (dd, 1H, *J* = 1.6 Hz, 10.4 Hz, CHH allyl), 5.20 (d, 1H, *J* = 3.8 Hz, H-1), 5.26 (dd, 1H, *J* = 1.7 Hz, 17.2 Hz, CHH allyl), 5.49 (s, 1H, CH benzylidene), 5.89 (ddd, 1H, *J* = 5.5 Hz, 10.7 Hz, 22.7 Hz, CH allyl), 6.83 (d, 2H, *J* = 8.6 Hz, H<sub>arom</sub>), 7.24 - 7.47 (m, 18H, H<sub>arom</sub>), 7.65 - 7.68 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR  $\alpha$ -product (100 MHz):  $\delta$  = 19.2 (C<sub>q</sub> *t*-Bu), 26.8 (3 x CH<sub>3</sub> TBDPS), 55.2 (OMe), 62.4 (C-5), 63.8 (CH<sub>2</sub> glycerol), 68.9 (C-6), 70.7 (CH<sub>2</sub> glycerol), 72.3 (CH<sub>2</sub> allyl), 72.3, 75.2 (CH<sub>2</sub> Bn, CH<sub>2</sub> PMB), 76.6, 78.2 (C-3, C-4), 78.6 (CH glycerol), 82.1 (C-2), 97.2 (C-1), 101.2 (CH benzylidene), 113.7 (CH<sub>arom</sub>), 116.9 (CH<sub>2</sub> allyl), 126.1 - 129.7 (CH<sub>arom</sub>), 130.4, 133.2, 133.2, 134.7, 135.5, 137.5, 138.9 (CH<sub>arom</sub>, CH allyl, 2 x C<sub>q</sub> phenyl, C<sub>q</sub> PMB, C<sub>q</sub> Bn, C<sub>q</sub> benzylidene), 159.2 (C<sub>q</sub> PMB); HRMS: C<sub>50</sub>H<sub>58</sub>O<sub>9</sub>Si + Na<sup>+</sup> requires 853.3742, found 853.3745.) A combination of batches of the fully protected pesudodisaccharide (8.81 g, 10.6 mmol) was dissolved in DCM (100 ml) and cooled to 0 °C. After adding H<sub>2</sub>O (5.0 ml) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ, 2.88 g, 12.7 mmol), respectively, the mixture was stirred vigorously for 4 h, before it was quenched by the addition of sat. aq. NaHCO<sub>3</sub> (25 ml). The mixture was diluted with EtOAc (500 ml) and washed with sat. aq. NaHCO<sub>3</sub> (3 x 200 ml) and brine (3 x 150 ml), respectively, in order to remove the bulk of DDQH<sub>2</sub>. The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/PE). Alcohol **14** (4.23 g, 5.95 mmol, 56%) was obtained as pale yellow oil.

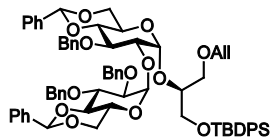
#### From **13b**:

A solution of donor **13b** (145 mg, 0.250 mmol), TTBP (155 mg, 0.625 mmol) and Ph<sub>2</sub>SO (60.7 mg, 0.300 mmol) in freshly distilled DCM (8.0 ml), together with activated MS 3Å, was stirred under argon at RT for 30 min. Subsequently, the mixture was cooled to -78 °C and stirred for another 15 min. After the addition of Tf<sub>2</sub>O (50.5  $\mu$ l, 0.300 mmol), the mixture was stirred for 45 min at -50 °C before it was cooled down to -78 °C. After the addition of a solution of glycerol acceptor **7** (111 mg, 0.300 mmol) in DCM (2.5 ml), the mixture was stirred at -75 °C for 1h, before it was allowed to gradually (~3 h) warm to room temperature. The reaction was quenched by the addition of Et<sub>3</sub>N (0.2 ml, 1.4 mmol) and stirred for 30 min. The mixture was diluted with DCM (30 ml) and after washing with sat. aq. NaHCO<sub>3</sub> (15 ml) and brine (15 ml), the organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. Purification of the resulting oil by size-exclusion chromatography (sephadex LH-20, MeOH/DCM 1/1), followed by column chromatography (EtOAc/PE) gave the fully protected intermediate (131 mg, 0.156 mmol, 62%,  $\alpha/\beta$  mixture, ~7/1), as pale yellow oil. (Analytical data: <sup>1</sup>H NMR  $\alpha$ -product (400 MHz):  $\delta$  = 1.06 (s, 9H, *t*-Bu), 3.49 - 4.04 (m, 13H, CH glycerol, 2 x CH<sub>2</sub> glycerol, H-2, H-3, H-4, H-5, 2 x H-6, CH<sub>2</sub> allyl), 4.68 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.74 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.80 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.88 (d, 1H, *J* = 11.6 Hz, CHH Bn), 5.17 (dd, 1H, *J* = 1.2 Hz, 10.4 Hz, CHH allyl), 5.23 - 5.28 (m, 2H, H-1, CHH allyl), 5.50 (s, 1H, CH benzylidene), 5.88 (ddd, 1H, *J* = 5.6 Hz, 10.6 Hz, 22.8

Hz, CH allyl), 6.93 (d, 2H,  $J$  = 8.4 Hz,  $H_{\text{arom}}$ ), 7.22 - 7.46 (m, 18H,  $H_{\text{arom}}$ ), 7.64 - 7.68 (m, 4H,  $H_{\text{arom}}$ );  $^{13}\text{C}$  NMR  $\alpha$ -product (100 MHz):  $\delta$  = 19.2 ( $C_q$  *t*-Bu, 26.8 (3  $\times$   $\text{CH}_3$  TBDPS), 62.4 (C-5), 63.9 ( $\text{CH}_2$  glycerol), 68.8 (C-6), 70.9 ( $\text{CH}_2$  glycerol), 71.8, 72.3, 75.2 ( $\text{CH}_2$  allyl,  $\text{CH}_2$  *p*- $\text{N}_3\text{Bn}$ ,  $\text{CH}_2$  Bn), 76.7, 78.2 (C-3, C-4), 79.0 (CH glycerol), 82.2 (C-2), 97.2 (C-1), 101.2 (CH benzylidene), 116.9 ( $\text{CH}_2$  allyl), 118.8 ( $\text{CH}_{\text{arom}}$ ) 126.1 - 129.7 ( $\text{CH}_{\text{arom}}$ ), 133.1, 133.2 (2  $\times$   $C_q$  phenyl), 134.6 (CH allyl), 135.1 ( $C_q$  *p*- $\text{N}_3\text{Bn}$ ), 135.5 ( $\text{CH}_{\text{arom}}$ ), 137.5, 138.8, 139.3 ( $C_q$  Bn,  $C_q$  benzylidene  $C_q$  *p*- $\text{N}_3\text{Bn}$ ). A part of the intermediate (58 mg, 0.069 mmol) was dissolved in a mixture of dioxane/water (9/1, 10 ml) and treated with  $\text{PMe}_3$  (1M solution in toluene, 0.34 ml). After stirring for 1h, the mixture was concentrated *in vacuo* and coevaporated three times with dioxane (portions of 5 ml) before the mixture was redissolved in moist DCM (10 ml) and DDQ (24 mg, 0.10 mmol) was added. After stirring vigorously for 4 h, the reaction was quenched by the addition of sat. aq.  $\text{NaHCO}_3$  (2.5 ml). The mixture was then diluted with EtOAc (40 ml) and washed with, respectively, sat. aq.  $\text{NaHCO}_3$  (3  $\times$  15 ml) and brine (3  $\times$  15 ml) in order to remove the bulk of DDQH<sub>2</sub>. The organic layer was dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The crude product was purified by column chromatography (EtOAc/PE), giving alcohol **14** (19.0 mg, 26.7  $\mu\text{mol}$ , 39%) as pale yellow oil.

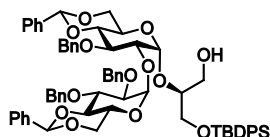
#### From **12** (via **13c**):

To a solution of thioglucose **12** (225 mg, 0.500 mmol) in DCM (5.0 ml) were added  $\text{Et}_3\text{N}$  (0.69 ml, 5.0 mmol), TMS-Cl (0.32 ml, 2.50 mmol) and a drop of pyridine. After stirring for 2 h, the reaction was diluted with EtOAc (40 ml) and washed with  $\text{H}_2\text{O}$  (15 ml) and brine (15 ml) respectively. The organic layer was dried with  $\text{MgSO}_4$  and concentrated *in vacuo*. Crude **13c**, together with  $\text{Ph}_2\text{SO}$  (121 mg, 0.600 mmol) and TTBP (323 mg, 1.30 mmol) were coevaporated with toluene (3  $\times$  5 ml) and together with activated  $\text{MS3\AA}$  dissolved in dry DCM (10 ml). After stirring for 15 min, the mixture was cooled to  $-78^\circ\text{C}$  and stirred for 5 min. After the addition of  $\text{Tf}_2\text{O}$  (101  $\mu\text{l}$ , 0.600 mmol), the mixture was allowed to slowly warm up to  $-60^\circ\text{C}$  ( $\sim 30$  min), after which the mixture was cooled down to  $-78^\circ\text{C}$  and glycerol acceptor **7** (278 mg, 0.750 mmol, dissolved in 2.0 ml DCM) was added. After stirring for 1 h at  $-78^\circ\text{C}$ , the mixture was allowed to warm up to RT overnight. After the addition of a few drops of  $\text{H}_2\text{O}$  the mixture was diluted with MeOH (20 ml).  $\text{K}_2\text{CO}_3$  (10 g) was subsequently added and, after stirring for 5 hrs, the mixture was diluted with EtOAc (100 ml). The organic layer was washed with  $\text{H}_2\text{O}$  (40 ml) and brine (40 ml) and dried with  $\text{MgSO}_4$ . After removal of the solvents *in vacuo*, the residue was purified by column chromatography (EtOAc/PE), affording **14** (165 mg, 0.232 mmol, 46%, only  $\alpha$ ) as pale yellow oil.  $[\alpha]_{\text{D}^{20}}(\text{CHCl}_3)$ : +33.6; IR: 741, 1040, 1072, 2343, 2361  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.06 (s, 9H, *t*-Bu), 2.92 (bs, 1H, OH), 3.54 - 3.79 (m, 8H, 2  $\times$   $\text{CH}_2$  glycerol, H-2, H-3, H-4, H-6), 3.85 - 3.95 (m, 2H, H-5, CH glycerol), 4.00 (ad, 2H,  $J$  = 5.6 Hz,  $\text{CH}_2$  allyl), 4.05 (dd, 1H,  $J$  = 4.9 Hz, 10.2 Hz, H-6), 4.85 (d, 1H,  $J$  = 11.8 Hz, CHH Bn), 4.91 (d, 1H,  $J$  = 11.8 Hz, CHH Bn), 5.06 (d, 1H,  $J$  = 3.8 Hz, H-1), 5.20 (dd, 1H,  $J$  = 1.1 Hz, 10.4 Hz, CHH allyl), 5.28 (dd, 1H,  $J$  = 1.5 Hz, 17.2 Hz, CHH allyl), 5.51 (s, 1H, CH benzylidene), 5.88 (ddd, 1H,  $J$  = 5.5 Hz, 10.8 Hz, 22.8 Hz, CH allyl), 7.23 - 7.47 (m, 16H,  $H_{\text{arom}}$ ), 7.64 - 7.68 (m, 4H,  $H_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 19.2 ( $C_q$  *t*-Bu), 26.8 (3  $\times$   $\text{CH}_3$  TBDPS), 63.0 (C-5), 63.8 ( $\text{CH}_2$  glycerol), 68.8 (C-6), 69.8 ( $\text{CH}_2$  glycerol), 72.3 ( $\text{CH}_2$  allyl), 73.1 (C-2), 74.6 ( $\text{CH}_2$  Bn), 79.0 (CH glycerol), 79.1, 81.5 (C-3, C-4), 99.8 (C-1), 101.2 (CH benzylidene), 117.5 ( $\text{CH}_2$  allyl), 126.1 - 129.8 ( $\text{CH}_{\text{arom}}$ ), 133.0, 133.1 (2  $\times$   $C_q$  phenyl), 134.2 (CH allyl), 135.5 ( $\text{CH}_{\text{arom}}$ ), 137.5, 138.8 ( $C_q$  Bn,  $C_q$  benzylidene); HRMS:  $\text{C}_{42}\text{H}_{50}\text{O}_8\text{Si} + \text{Na}^+$  requires 734.3201, found 734.3203.



**1-O-(tert-Butyldiphenylsilyl)-2-O-(2-[2,3-di-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranosyl]-3-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranosyl)-3-O-allyl-sn-glycerol (16)**

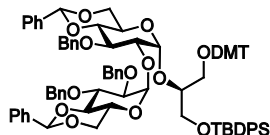
A solution of glucose donor **15** (130 mg, 0.240 mmol), TTBP (149 mg, 0.600 mmol) and Ph<sub>2</sub>SO (57 mg, 0.28 mmol) in freshly distilled DCM (5.0 ml), together with activated MS3Å, was stirred under argon at RT for 30 min. The mixture was cooled to -78 °C and stirred for another 15 min before Tf<sub>2</sub>O (47  $\mu$ l, 0.28 mmol) was added and the mixture stirred for another 45 min at -70 °C. Subsequently, alcohol **14** (142 mg, 0.200 mmol, dissolved in 2.5 ml DCM) was added and after stirring for 60 min at -75 °C, the mixture was allowed to, gradually, warm to room temperature (~3 h). The reaction was quenched by the addition of Et<sub>3</sub>N (0.14 ml, 1.0 mmol) and stirred for 30 min. The mixture was diluted with EtOAc (30 ml) and washed with sat. aq. NaHCO<sub>3</sub> (10 ml) and brine (10 ml). The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. The resulting oil was purified by size-exclusion chromatography (sephadex LH-20, MeOH/DCM 1/1) and, subsequently, column chromatography (EtOAc/pentane). Kojibiose derivative **16** (187 mg, 0.164 mmol, 82%, pure  $\alpha$ ) was obtained as white foam. [ $\alpha$ ]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +34.4; IR: 746, 997, 1028, 1074, 1369, 1454, 2860 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 1.05 (s, 9H, *t*-Bu), 3.54 (dd, 1H, *J* = 5.9 Hz, 10.5 Hz, CHH glycerol), 3.57 - 3.69 (m, 7H, 2 x CHH glycerol, H-2, 2 x H-4, 2 x H-6), 3.74 (dd, 1H, *J* = 5.3 Hz, 10.4 Hz, CHH glycerol), 3.80 (dd, 1H, *J* = 3.7 Hz, 9.4 Hz, H-2), 3.88 (m, 2H, CH<sub>2</sub> allyl), 3.91 - 4.00 (m, 2H, CH glycerol, H-5), 4.04 - 4.21 (m, 5H, 2 x H-3, H-5, 2 x H-6), 4.70 (d, 1H, *J* = 11.8 Hz, CHH Bn), 4.74 - 4.83 (m, 4H, CH<sub>2</sub> Bn), 4.90 (d, 1H, *J* = 11.8 Hz, CHH Bn), 5.12 (dd, 1H, *J* = 1.1 Hz, 10.4 Hz, CHH allyl), 5.19 (dd, 1H, *J* = 1.6 Hz, 17.3 Hz, CHH allyl), 5.27 (d, 1H, *J* = 3.5 Hz, H-1), 5.40 (d, 1H, *J* = 3.6 Hz, H-1), 5.52 (s, 1H, CH benzylidene), 5.54 (s, 1H, CH benzylidene), 5.82 (ddd, 1H, *J* = 5.6 Hz, 10.7 Hz, 22.7 Hz, CH allyl), 7.06 - 7.11 (m, 2H, H<sub>arom</sub>), 7.19 - 7.39 (m, 25H, H<sub>arom</sub>), 7.44 - 7.48 (m, 4H, H<sub>arom</sub>), 7.64 - 7.68 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 19.1 (C<sub>q</sub> *t*-Bu), 26.8 (3 x CH<sub>3</sub> TBDPS), 62.5 (C-5), 62.8 (C-5), 63.5 (CH<sub>2</sub> glycerol), 68.8 (C-6), 68.9 (C-6), 69.7 (CH<sub>2</sub> glycerol), 72.1 (CH<sub>2</sub> allyl), 72.4 (CH<sub>2</sub> Bn), 75.0 (CH<sub>2</sub> Bn), 75.3 (C-2), 75.3 (CH<sub>2</sub> Bn), 75.9 (CH glycerol), 76.7 (C-3), 78.1 (C-3), 79.0 (C-2), 82.2 (2 x C-4), 95.4 (C-1), 95.5 (C-1), 101.2 (2 x CH benzylidene), 117.0 (CH<sub>2</sub> allyl), 126.1 - 129.7 (CH<sub>arom</sub>), 133.2 (2 x C<sub>q</sub> phenyl), 134.5 (CH allyl), 135.5 (CH<sub>arom</sub>), 137.5, 137.6, 138.3, 138.6 (3 x C<sub>q</sub> Bn, 2 x C<sub>q</sub> benzylidene); HRMS: C<sub>69</sub>H<sub>76</sub>O<sub>13</sub>Si + Na<sup>+</sup> requires 1163.4947, found 1163.4962.



**1-O-(tert-Butyldiphenylsilyl)-2-O-(2-[2,3-di-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranosyl]-3-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranosyl)-sn-glycerol (17)**

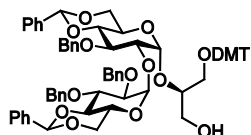
A solution of **16** (3.18 g, 2.79 mmol) and freshly activated MS3Å in freshly distilled THF (35 ml) was stirred under argon for 30 min. After the addition of Ir(COD)(Ph<sub>2</sub>MeP)<sub>2</sub>PF<sub>6</sub> (236 mg, 10 mol %) the solution turned red and the mixture was purged with H<sub>2</sub> (g) for ~ 1 min. After stirring under argon for 4 h, the solution was diluted with THF (30 ml) and sat. aq. NaHCO<sub>3</sub> (100 ml). After the addition of I<sub>2</sub> (1.06 g, 4.19 mmol), the mixture was allowed to stir overnight at room temperature. The mixture was diluted with EtOAc (250 ml) and washed with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (2 x 80 ml) and brine (80 ml), respectively. The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. Column chromatography (EtOAc/toluene) afforded **17** (2.05 g, 1.86 mmol, 67%) as pale yellow foam. [ $\alpha$ ]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +29.4; IR: 748, 997, 1028, 1074, 1371, 2360, 2858 cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 1.07 (s, 9H, *t*-Bu), 3.44 (dd, 1H, *J* = 5.3 Hz, 8.1 Hz, OH), 3.50 - 3.68 (m, 8H, 2 x CHH glycerol, 2 x H-2, 2 x H-4, 2 x H-6), 3.76 - 3.91 (m, 5H, 2 x CHH glycerol, CH glycerol, H-5, H-6), 3.96 (dd, 1H, *J* = 4.9 Hz, 10.2 Hz, H-6), 4.02 (at, 1H, *J* = 9.3 Hz, H-3), 4.16 (at, 1H, *J* = 9.3 Hz, H-3), 4.13 - 4.22 (m, 1H, H-5), 4.73 - 4.86 (m, 5H, H-1, 2 x CH<sub>2</sub> Bn), 4.94 (d, 1H, *J* = 11.1 Hz, CHH Bn), 4.97 (d, 1H, *J* = 10.7 Hz, CHH Bn), 5.09 (d, 1H, *J* = 3.6 Hz, H-1), 5.48 (s, 1H, CH

benzylidene), 5.50 (s, 1H, CH benzylidene), 7.12 - 7.15 (m, 3H,  $H_{\text{arom}}$ ), 7.27 - 7.47 (m, 28H,  $H_{\text{arom}}$ ), 7.66 - 7.68 (m, 4H,  $H_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 19.1 ( $\text{C}_q$  *t*-Bu), 26.8 (3 x  $\text{CH}_3$  TBDPS), 62.4 ( $\text{CH}_2$  glycerol), 62.5 (C-5), 62.8 (C-5), 63.7 ( $\text{CH}_2$  glycerol), 68.7 (2 x C-6), 74.0 ( $\text{CH}_2$  Bn), 75.1 ( $\text{CH}_2$  Bn), 75.2 ( $\text{CH}_2$  Bn), 76.4 (C-3), 77.2 (C-2), 77.7 (C-2), 78.8 (C-3), 80.7 (CH glycerol), 78.1 (C-3), 79.0 (C-2), 82.5 (C-4), 82.8 (C-4), 97.6 (C-1), 98.6 (C-1), 101.2 (CH benzylidene), 101.3 (CH benzylidene), 126.0 - 129.8 ( $\text{CH}_{\text{arom}}$ ), 133.0, 133.1 (2 x  $\text{C}_q$  phenyl), 135.5 ( $\text{CH}_{\text{arom}}$ ), 137.2, 137.5, 137.6, 138.0, 138.5 (3 x  $\text{C}_q$  Bn, 2 x  $\text{C}_q$  benzylidene); HRMS:  $\text{C}_{66}\text{H}_{72}\text{O}_{13}\text{Si}$  +  $\text{Na}^+$  requires 1123.4634, found 1123.4648.



**1-O-(*tert*-Butyldiphenylsilyl)-2-O-(2-[2,3-di-*O*-benzyl-4,6-*O*-benzylidene- $\alpha$ -D-glucopyranosyl]-3-*O*-benzyl-4,6-*O*-benzylidene- $\alpha$ -D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-sn-glycerol (18)**

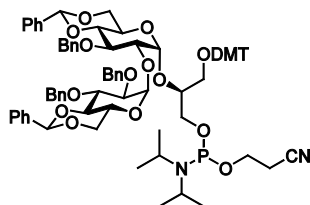
Alcohol **17** (480 mg, 0.436 mmol) was dissolved in DCM (20 ml) and cooled to 0 °C. After the addition of DIPEA (0.23 ml, 1.3 mmol) and DMT-Cl (295 mg, 0.872 mmol) respectively, the mixture was allowed to stir at room temperature overnight. The reaction was quenched upon addition of  $\text{H}_2\text{O}$  (0.2 ml), and stirred for 15 min. The mixture was diluted with DCM (20 ml) and, after washing with  $\text{H}_2\text{O}$  (10 ml) and brine (10 ml), the organic layer was dried over  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*. The resulting oil was purified by size-exclusion chromatography (sephadex LH-20, MeOH/DCM 1/1) and, subsequently, column chromatography (EtOAc/pentane/ $\text{Et}_3\text{N}$ ), giving DMTr-ether **18** (601 mg, 0.428 mmol, 98%) as white foam.  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): + 35.6; IR: 752, 1030, 1074, 1250, 1508, 2361, 2858  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 0.97 (s, 9H, *t*-Bu), 3.08 (dd, 1H,  $J$  = 5.7 Hz, 10.1 Hz, CHH glycerol), 3.44 (dd, 1H,  $J$  = 3.4 Hz, 9.2 Hz, H-2), 3.51 (dd, 1H,  $J$  = 2.9 Hz, 10.1 Hz, CHH glycerol), 3.55 - 3.71 (m, 12H, 2 x OMe, 2 x CHH glycerol, 2 x H-4, 2 x H-6), 3.77 (dd, 1H,  $J$  = 3.4 Hz, 9.4 Hz, H-2), 3.93 - 4.02 (m, 3H, CH glycerol, H-3, H-5), 4.07 - 4.17 (m, 4H, H-3, H-5, 2 x H-6), 4.30 (d, 1H,  $J$  = 11.5 Hz, CHH Bn), 4.36 (d, 1H,  $J$  = 11.4 Hz, CHH Bn), 4.56 (d, 1H,  $J$  = 11.4 Hz, CHH Bn), 4.72 (d, 1H,  $J$  = 11.4 Hz, CHH Bn), 4.78 (d, 1H,  $J$  = 11.0 Hz, CHH Bn), 4.88 (d, 1H,  $J$  = 11.0 Hz, CHH Bn), 4.98 (d, 1H,  $J$  = 3.4 Hz, H-1), 5.39 (d, 1H,  $J$  = 3.4 Hz, H-1), 5.53 (s, 1H, CH benzylidene), 5.55 (s, 1H, CH benzylidene), 6.74 (d, 4H,  $J$  = 8.7 Hz,  $H_{\text{arom}}$ ), 7.08 - 7.48 (m, 40H,  $H_{\text{arom}}$ ), 7.55 (d, 2H,  $J$  = 7.1 Hz,  $H_{\text{arom}}$ ), 7.59 (d, 2H,  $J$  = 6.7 Hz,  $H_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 19.1 ( $\text{C}_q$  *t*-Bu), 26.8 (3 x  $\text{CH}_3$  TBDPS), 55.1 (2 x OMe), 62.5 (C-5), 62.8 (C-5), 63.3 ( $\text{CH}_2$  glycerol), 64.3 ( $\text{CH}_2$  glycerol), 68.8 (C-6), 68.9 (C-6), 72.4 ( $\text{CH}_2$  Bn), 75.0 ( $\text{CH}_2$  Bn), 75.4 ( $\text{CH}_2$  Bn), 75.7 (C-2), 76.8, 76.9 (C-3, CH glycerol), 78.1 (C-3), 79.4 (C-2), 82.1 (C-4), 82.3 (C-4), 86.4 ( $\text{C}_q$  DMT), 95.2 (C-1), 95.6 (C-1), 101.2 (CH benzylidene), 101.3 (CH benzylidene), 113.1 ( $\text{CH}_{\text{arom}}$ ), 126.1 - 130.0 ( $\text{CH}_{\text{arom}}$ ), 133.1, 133.2 (2 x  $\text{C}_q$  phenyl), 135.5 ( $\text{CH}_{\text{arom}}$ ), 137.5, 137.7, 138.1, 138.2, 138.7, 144.9, 158.4 (3 x  $\text{C}_q$  Bn, 2 x  $\text{C}_q$  benzylidene, 5 x  $\text{C}_q$  DMT); HRMS:  $\text{C}_{87}\text{H}_{90}\text{O}_{15}\text{Si}$  +  $\text{Na}^+$  requires 1425.5941, found 1425.5961.



**2-O-(2-[2,3-Di-*O*-benzyl-4,6-*O*-benzylidene- $\alpha$ -D-glucopyranosyl]-3-*O*-benzyl-4,6-*O*-benzylidene- $\alpha$ -D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-sn-glycerol (19)**

To a solution of kojibiose derivative **18** (575 mg, 0.410 mmol) in THF (10 ml), was added TBAF (1.00 M solution in THF, 1.20 ml). After stirring for 48 h at RT, the solvent was removed *in vacuo*. The residue was purified by column chromatography (EtOAc/pentane/ $\text{Et}_3\text{N}$ ), affording compound **19** (435 mg, 0.373 mmol, 91%) as white foam.  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): + 38.0; IR: 1030, 1074, 1508, 2341, 2361  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 2.52 (bs, 1H, OH), 3.02 (dd, 1H,  $J$  = 4.1 Hz, 9.9 Hz, CHH glycerol), 3.44 - 3.49 (m, 2H, CHH glycerol, H-2), 3.55 - 3.78 (m, 14H, 2 x OMe, 2 x CHH glycerol, CH glycerol, H-2, 2 x H-4, 2 x H-6), 3.96 (at, 1H,  $J$  = 9.3 Hz, H-3), 4.04 - 4.16 (m, 4H, H-3, 2 x H-5, H-6), 4.31 (dd, 1H,  $J$  = 4.8 Hz, 10.2 Hz, H-6), 4.49 - 4.55 (m, 3H, 3 x CHH Bn), 4.73 (d, 1H,  $J$  = 11.4

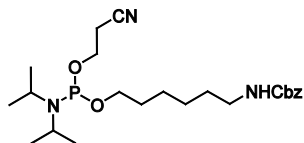
Hz, CHH Bn), 4.83 (d, 1H,  $J$  = 11.0 Hz, CHH Bn), 4.90 (d, 1H,  $J$  = 3.3 Hz, H-1), 4.93 (d, 1H,  $J$  = 11.0 Hz, CHH Bn), 5.17 (d, 1H,  $J$  = 3.5 Hz, H-1), 5.52 (s, 1H, CH benzylidene), 5.58 (s, 1H, CH benzylidene), 6.75 (d, 2H,  $J$  = 8.8 Hz,  $H_{\text{arom}}$ ), 6.76 (d, 2H,  $J$  = 8.8 Hz,  $H_{\text{arom}}$ ), 7.16 - 7.50 (m, 34H,  $H_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 55.1 (2 x OMe), 63.0 (2 x C-5), 63.8 (CH<sub>2</sub> glycerol), 64.3 (CH<sub>2</sub> glycerol), 68.7 (C-6), 68.9 (C-6), 73.5 (CH<sub>2</sub> Bn), 75.0 (CH<sub>2</sub> Bn), 75.4 (CH<sub>2</sub> Bn), 76.9 (C-3), 77.0 (C-2), 78.4 (C-3), 79.0 (C-2), 79.8 (CH glycerol), 82.3 (C-4), 82.5 (C-4), 86.3 (C<sub>q</sub> DMTr), 97.1 (C-1), 97.2 (C-1), 101.2 (CH benzylidene), 101.3 (CH benzylidene), 113.1 (CH<sub>arom</sub>), 126.0 - 129.9 (CH<sub>arom</sub>), 135.7, 135.9, 137.2, 137.6, 137.8, 138.1, 138.6, 144.7, 158.4 (3 x C<sub>q</sub> Bn, 2 x C<sub>q</sub> benzylidene, 5 x C<sub>q</sub> DMTr); HRMS: C<sub>71</sub>H<sub>72</sub>O<sub>15</sub> + Na<sup>+</sup> requires 1187.4763, found 1187.4782.



**1-([N,N-diisopropylamino]-2-cyanoethyl-phosphite)-2-O-(2-[2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl]-3-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-sn-glycerol (4)**

To a cooled (0 °C) solution of alcohol **19** (1.37 g, 1.17 mmol) and DIPEA (0.31 ml, 1.75 mmol) in freshly distilled DCM (12 ml) was added chlorophosphite **11** (333 mg, 1.41 mmol). After stirring for 4 h, the reaction was quenched with H<sub>2</sub>O (1.0 ml),

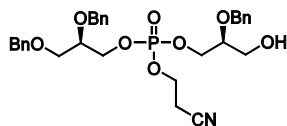
diluted with DCM (50 ml) and washed with H<sub>2</sub>O (20 ml) and brine (20 ml) respectively. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. After purification of the residue by column chromatography (EtOAc/pentane/Et<sub>3</sub>N), phosphoramidite **4** (1.15 g, 0.842 mmol, 72%) was obtained as colourless oil.  $^{31}\text{P}$  NMR (161.7 MHz, CD<sub>3</sub>CN):  $\delta$  = 148.5, 148.7 (diastereoisomers);  $^1\text{H}$  NMR (400 MHz, CD<sub>3</sub>CN):  $\delta$  = 1.10 - 1.17 (m, 12H, CH<sub>3</sub> isopropylamino), 2.46 - 2.52 (m, 2H, CH<sub>2</sub> cyanoethyl), 3.12 - 3.16 (m, 1H, CH isopropylamino), 3.32 - 3.39 (m, 1H, CH isopropylamino), 3.51 - 4.15 (m, 24H, 2 x OMe, 2 x CH<sub>2</sub> glycerol, CH glycerol, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 3 x H-6, CH<sub>2</sub> cyanoethyl), 4.29 (dd, 1H,  $J$  = 4.2 Hz, 10.0 Hz, H-6), 4.39 (d, 1H,  $J$  = 11.4 Hz, CHH Bn), 4.46 (d, 1H,  $J$  = 11.3 Hz, CHH Bn), 4.60 - 4.70 (m, 2H, 2 x CHH Bn), 4.81 (s, 2H, 2 x CHH Bn), 5.12 (d, 1H,  $J$  = 3.3 Hz, H-1), 5.30 (d, 0.5H,  $J$  = 3.3 Hz, H-1 diastereomer 1), 5.34 (d, 0.5H,  $J$  = 3.3 Hz, H-1 diastereomer 2), 5.60 (s, 1H, CH benzylidene), 5.66 (s, 1H, CH benzylidene), 6.82 (d, 4H,  $J$  = 8.6 Hz,  $H_{\text{arom}}$ ), 7.16 - 7.50 (m, 34H,  $H_{\text{arom}}$ ); TLC-MS: C<sub>80</sub>H<sub>89</sub>N<sub>2</sub>O<sub>16</sub>P + H<sup>+</sup> requires 1365.60, found 1365.6.



**benzyl 6-([N,N-diisopropylamino]-2-cyanoethyl-phosphite)-hexyl-1-carbamate (5)**

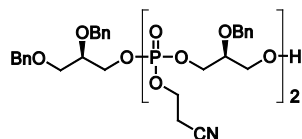
To a cooled (0 °C) solution of 6-(benzyloxycarbonylamino)-1-hexanol<sup>41</sup> (3.02 g, 12.0 mmol) and DIPEA (2.51 ml, 14.4 mmol) in DCM (60 ml) was added chlorophosphite **11** (2.90 g, 12.2 mmol). After stirring for 4 h, the reaction was washed with H<sub>2</sub>O

(20 ml) and brine (20 ml), respectively. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. After purification of the residue by column chromatography (EtOAc/PE/Et<sub>3</sub>N), phosphoramidite **5** (3.97 g, 8.79 mmol, 73%) was obtained as colourless oil.  $^{31}\text{P}$  NMR (161.7 MHz, CD<sub>3</sub>CN):  $\delta$  = 148.2;  $^1\text{H}$  NMR (400 MHz, CD<sub>3</sub>CN):  $\delta$  = 1.18 (d, 6H,  $J$  = 2.7 Hz, 2 x CH<sub>3</sub> isopropylamino), 1.19 (d, 6H,  $J$  = 2.7 Hz, 2 x CH<sub>3</sub> isopropylamino), 1.30 - 1.43 (m, 4H, 2 x CH<sub>2</sub> aminohexanol), 1.48 (m, 2H, CH<sub>2</sub> aminohexanol), 1.59 (m, 2H, CH<sub>2</sub> aminohexanol), 2.65 (t, 2H,  $J$  = 6.0 Hz, CH<sub>2</sub> cyanoethyl), 3.10 (dd, 2H,  $J$  = 6.7 Hz, 13.2 Hz, CH<sub>2</sub>-N aminohexanol), 3.57 - 3.68 (m, 4H, CH<sub>2</sub>-O aminohexanol), 2 x CH isopropylamino), 3.73 - 3.85 (m, 2H, CH<sub>2</sub> cyanoethyl), 5.06 (bs, 2H, CH<sub>2</sub> benzylcarbamate), 5.64 (bs, 1H, NH), 7.31 - 7.42 (m, 5H,  $H_{\text{arom}}$ ); TLC-MS: C<sub>23</sub>H<sub>38</sub>N<sub>3</sub>O<sub>4</sub>P + H<sup>+</sup> requires 452.27, found 452.1.



### Glycerol phosphate dimer (21)

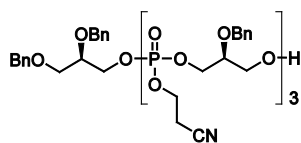
To a solution of dibenzylglycerol **2** (163 mg, 0.600 mmol) in acetonitrile (4.0 ml), containing freshly activated MS3Å, were added DCI (0.25 M in acetonitrile, 9.6 ml) and phosphoramidite **3** (0.2 M in acetonitrile, 3.9 ml). After stirring for 2 h, I<sub>2</sub> (0.2 M in THF/pyridine 4/1, 9.0 ml) and H<sub>2</sub>O (1.0 ml) were added and the mixture was allowed to stir for 1 h. EtOAc (80 ml) was added and the mixture was washed with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (30 ml), aqueous KHSO<sub>4</sub> (0.5 M, 30 ml), sat. aq. NaHCO<sub>3</sub> (30 ml) and brine (30 ml) respectively. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*, after which the residue was redissolved in DCM (30 ml), containing freshly activated MS3Å. Et<sub>3</sub>SiH (0.97 ml, 6.0 mmol) and dichloroacetic acid (0.49 ml, 6.0 mmol) were added and the mixture stirred until the orange colour disappeared (~1 h), after which the mixture was diluted with DCM (20 ml) and washed with sat. aq. NaHCO<sub>3</sub> (20 ml) and brine (20 ml), respectively. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, concentrated *in vacuo* and subsequently purified by column chromatography (MeOH/DCM), yielding dimer **21** (272 mg, 0.478 mmol, 80%, mixture of diastereoisomers) as colourless oil. <sup>31</sup>P NMR (161.7 MHz):  $\delta$  = -0.6, -0.7; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 2.50 (t, 1H, *J* = 6.2 Hz, CH<sub>2</sub> cyanoethyl), 2.54 (t, 1H, *J* = 6.2 Hz, CH<sub>2</sub> cyanoethyl), 2.66 (bs, 1H, OH), 3.54 - 3.73 (m, 5H, CH glycerol, 2 x CH<sub>2</sub> glycerol), 3.77 - 3.82 (m, 1H, CH glycerol), 4.03 - 4.32 (m, 6H, CH<sub>2</sub> cyanoethyl, 2 x CH<sub>2</sub> glycerol), 4.51 - 4.66 (m, 6H, 3 x CH<sub>2</sub> Bn), 7.25 - 7.35 (m, 15H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 19.2, 19.3, 19.3 (CH<sub>2</sub> cyanoethyl), 60.6, 60.7 (CH<sub>2</sub> glycerol), 61.9 (CH<sub>2</sub> cyanoethyl), 66.3, 66.4 (CH<sub>2</sub> glycerol), 67.4, 67.5, 67.6 (CH<sub>2</sub> glycerol), 68.6 (CH<sub>2</sub> glycerol), 71.9, 72.0 (CH<sub>2</sub> Bn), 72.1 (CH<sub>2</sub> Bn), 73.4 (CH<sub>2</sub> Bn), 76.2, 76.3 (CH glycerol), 77.3, 77.4, 77.5 (CH glycerol), 116.4 (C<sub>q</sub> cyanoethyl), 127.6 - 128.4 (CH<sub>arom</sub>), 137.7, 137.8, 137.8 (C<sub>q</sub> Bn); HRMS: C<sub>30</sub>H<sub>37</sub>NO<sub>8</sub>P + Na<sup>+</sup> requires 592.2071, found 592.2070.



### Glycerol phosphate trimer (22)

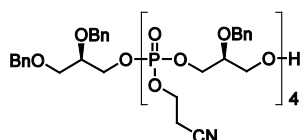
To a solution of dimer **21** (229 mg, 0.402 mmol) in acetonitrile (4.0 ml), containing freshly activated MS3Å, were added DCI (0.25 M in acetonitrile, 6.4 ml) and phosphoramidite **3** (0.2 M in acetonitrile, 2.6 ml). After stirring for 2 h, more **3** (0.2 M in acetonitrile, 0.8 ml) was added and the mixture was allowed to react for another 2 hrs. I<sub>2</sub> (0.2 M in THF/pyridine 4/1, 6.0 ml) and H<sub>2</sub>O (1.0 ml) were added and the mixture was stirred for 1 h. EtOAc (50 ml) was added and the mixture was washed with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (20 ml), aqueous KHSO<sub>4</sub> (0.5 M, 20 ml), sat. aq. NaHCO<sub>3</sub> (20 ml) and brine (20 ml) respectively. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*, after which the residue was redissolved in DCM (20 ml), containing freshly activated MS3Å. Et<sub>3</sub>SiH (0.65 ml, 4.0 mmol) and dichloroacetic acid (0.32 ml, 4.0 mmol) were added and the mixture stirred until the orange colour disappeared (~1.5 h), after which the mixture was diluted with DCM (30 ml) and washed with sat. aq. NaHCO<sub>3</sub> (20 ml) and brine (20 ml) respectively. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, concentrated *in vacuo* and the residue purified by column chromatography (MeOH/DCM), yielding trimer **22** (286 mg, 0.330 mmol, 82%) as colourless oil. <sup>31</sup>P NMR (161.7 MHz):  $\delta$  = -1.2, -1.2, -1.1, -1.0, -0.8, -0.8; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 2.47 - 2.58 (m, 4H, 2 x CH<sub>2</sub> cyanoethyl), 2.97 (bs, 1H, OH), 3.53 - 3.80 (m, 7H, 3 x CH glycerol, 2 x CH<sub>2</sub> glycerol), 4.03 - 4.32 (m, 12H, 2 x CH<sub>2</sub> cyanoethyl, 4 x CH<sub>2</sub> glycerol), 4.48 - 4.66 (m, 8H, 4 x CH<sub>2</sub> Bn), 7.25 - 7.33 (m, 20H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 19.2 - 19.4 (CH<sub>2</sub> cyanoethyl), 60.5, 60.6 (CH<sub>2</sub> glycerol), 61.8 - 62.1 (2 x CH<sub>2</sub> cyanoethyl), 65.5 - 65.9 (2 x CH<sub>2</sub> glycerol), 66.5 - 66.7 (CH<sub>2</sub> glycerol), 67.4 - 67.7 (CH<sub>2</sub> glycerol), 68.6 - 68.7 (CH<sub>2</sub> glycerol), 71.9 - 72.1 (3 x CH<sub>2</sub> Bn), 73.4 (CH<sub>2</sub> Bn), 75.3 - 75.5 (CH glycerol), 76.3 - 76.4 (CH glycerol), 77.4 - 77.6 (CH glycerol), 116.4 - 116.5 (2 x C<sub>q</sub> cyanoethyl), 127.6 - 128.4 (CH<sub>arom</sub>), 137.2, 137.7 - 137.8 (4 x C<sub>q</sub> Bn); HRMS: C<sub>43</sub>H<sub>52</sub>N<sub>2</sub>O<sub>13</sub>P<sub>2</sub> + Na<sup>+</sup> requires 889.2837, found 889.2843.





### Glycerol phosphate tetramer (23)

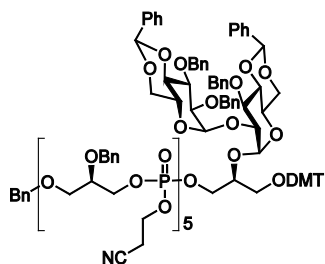
To a solution of trimer **22** (280 mg, 0.323 mmol) in acetonitrile (3.0 ml), containing freshly activated MS3Å, were added DCI (0.25 M in acetonitrile, 5.2 ml) and phosphoramidite **3** (0.2 M in acetonitrile, 2.6 ml). After stirring for 2 h, more **3** (0.2 M in acetonitrile, 1.3 ml) was added and the mixture was allowed to react for another 2 h. I<sub>2</sub> (0.2 M in THF/pyridine 4/1, 6.5 ml) and H<sub>2</sub>O (1.0 ml) were added and the mixture was stirred for 1 h. EtOAc (50 ml) was added and the mixture was washed with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (20 ml), aqueous KHSO<sub>4</sub> (0.5 M, 20 ml), sat. aq. NaHCO<sub>3</sub> (20 ml) and brine (20 ml) respectively. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*, after which the residue was redissolved in DCM (15 ml), containing freshly activated MS3Å. Et<sub>3</sub>SiH (0.52 ml, 3.23 mmol) and dichloroacetic acid (0.27 ml, 3.23 mmol) were added and the mixture stirred until the orange colour disappeared (~2 h), after which the mixture was diluted with DCM (20 ml) and washed with sat. aq. NaHCO<sub>3</sub> (15 ml) and brine (15 ml) respectively. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, concentrated *in vacuo* and, subsequently, purified by column chromatography (MeOH/DCM), yielding tetramer **23** (305 mg, 0.262 mmol, 81%) as colourless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.3 - -0.8; <sup>1</sup>H NMR (400 MHz): δ = 2.47 - 2.61 (m, 6H, 3 x CH<sub>2</sub> cyanoethyl), 3.20 (bs, 1H, OH), 3.56 - 3.58 (m, 2H, CH<sub>2</sub> glycerol) 3.62 - 3.71 (m, 3H, CH glycerol, CH<sub>2</sub> glycerol), 3.78 - 3.81 (m, 3H, 3 x CH glycerol), 4.03 - 4.30 (m, 18H, 3 x CH<sub>2</sub> cyanoethyl, 6 x CH<sub>2</sub> glycerol), 4.50 - 4.65 (m, 10H, 5 x CH<sub>2</sub> Bn), 7.26 - 7.34 (m, 25H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 19.2 - 19.4 (3 x CH<sub>2</sub> cyanoethyl), 60.4, 60.5 (CH<sub>2</sub> glycerol), 61.8 - 62.2 (3 x CH<sub>2</sub> cyanoethyl), 65.5 - 66.0 (4 x CH<sub>2</sub> glycerol), 66.5 - 66.7 (CH<sub>2</sub> glycerol), 67.4 - 67.7 (CH<sub>2</sub> glycerol), 68.6 (CH<sub>2</sub> glycerol), 71.9 - 72.1 (4 x CH<sub>2</sub> Bn), 73.4 (CH<sub>2</sub> Bn), 75.2 - 75.5 (2 x CH glycerol), 76.2 - 76.4 (CH glycerol), 77.4 - 77.6 (CH glycerol), 116.5 (3 x C<sub>q</sub> cyanoethyl), 127.6 - 128.4 (CH<sub>arom</sub>), 137.2, 137.7 - 137.9 (5 x C<sub>q</sub> Bn); HRMS: C<sub>56</sub>H<sub>68</sub>N<sub>3</sub>O<sub>18</sub>P<sub>3</sub> + Na<sup>+</sup> requires 1186.3603, found 1186.3614.



### Glycerol phosphate pentamer (24)

To a solution of tetramer **23** (283 mg, 0.243 mmol) in acetonitrile (3.0 ml), containing freshly activated MS3Å, were added DCI (0.25 M in acetonitrile, 4.0 ml) and phosphoramidite **3** (0.2 M in acetonitrile, 2.5 ml). After stirring for 4 h, more **3** (0.2 M in acetonitrile, 1.2 ml) was added and the mixture was allowed to react overnight. I<sub>2</sub> (0.2 M in THF/pyridine 4/1, 5.0 ml) and H<sub>2</sub>O (1.0 ml) were added and the mixture was stirred for 1 h. EtOAc (30 ml) was added and the mixture was washed with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (15 ml), aqueous KHSO<sub>4</sub> (0.5 M, 15 ml), sat. aq. NaHCO<sub>3</sub> (15 ml) and brine (15 ml) respectively. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*, after which the residue was redissolved in DCM (15 ml), containing freshly activated MS3Å. Et<sub>3</sub>SiH (0.39 ml, 2.4 mmol) and dichloroacetic acid (0.20 ml, 2.4 mmol) were added and the mixture stirred until the orange colour disappeared (~3 h), after which the mixture was diluted with DCM (20 ml) and washed with, respectively, sat. aq. NaHCO<sub>3</sub> (15 ml) and brine (15 ml). The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, concentrated *in vacuo* and, subsequently, purified by column chromatography (MeOH/DCM), yielding pentamer **24** (221 mg, 0.151 mmol, 62%) as colourless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.3 - -0.8; <sup>1</sup>H NMR (400 MHz): δ = 1.67 (bs, 1H, OH), 2.45 - 2.64 (m, 8H, 4 x CH<sub>2</sub> cyanoethyl), 3.55 - 3.58 (m, 2H, CH<sub>2</sub> glycerol) 3.62 - 3.71 (m, 3H, CH glycerol, CH<sub>2</sub> glycerol), 3.75 - 3.80 (m, 4H, 4 x CH glycerol), 4.04 - 4.28 (m, 24H, 4 x CH<sub>2</sub> cyanoethyl, 8 x CH<sub>2</sub> glycerol), 4.49 - 4.51 (m, 2H, CH<sub>2</sub> Bn), 4.56 - 4.66 (m, 10H, 5 x CH<sub>2</sub> Bn), 7.26 - 7.35 (m, 30H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 19.2 - 19.4 (4 x CH<sub>2</sub> cyanoethyl), 60.2 - 60.4 (CH<sub>2</sub> glycerol), 62.0 - 62.5 (4 x CH<sub>2</sub> cyanoethyl), 65.6 - 66.1 (6 x CH<sub>2</sub> glycerol), 66.7 - 66.9 (CH<sub>2</sub> glycerol), 67.6 - 67.9 (CH<sub>2</sub> glycerol), 68.5 (CH<sub>2</sub> glycerol), 71.9 - 72.2 (5 x CH<sub>2</sub> Bn), 73.4 (CH<sub>2</sub> Bn), 75.2 - 75.4 (3 x CH glycerol), 76.3 - 76.4 (CH glycerol), 77.4 - 77.5 (CH glycerol), 116.6 - 116.8 (4

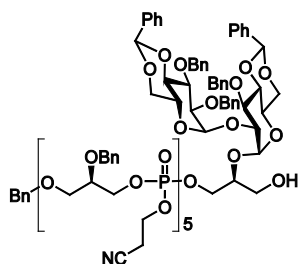
x C<sub>q</sub> cyanoethyl), 127.6 - 128.5 (C<sub>H<sub>arom</sub></sub>), 137.2 - 137.3, 137.7 - 137.8 (6 x C<sub>q</sub> Bn); HRMS: C<sub>69</sub>H<sub>84</sub>N<sub>4</sub>O<sub>23</sub>P<sub>4</sub> + Na<sup>+</sup> requires 1483.4369, found 1483.4377.



### Hexamer (25)

To a solution of pentamer **24** (150 mg, 0.103 mmol) and kojibiose-glycerol phosphoramidite **5** (350 mg, 0.257 mmol) in acetonitrile (3.0 ml), containing freshly activated MS3Å, was added DCI (0.25 M in acetonitrile, 2.0 ml). After stirring the mixture overnight at room temperature, I<sub>2</sub> (0.2 M in THF/pyridine 4/1, 2.5 ml) and H<sub>2</sub>O (1.0 ml) were added and the mixture was stirred for 1 h. The mixture was diluted with EtOAc (30 ml) and subsequently washed with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (10 ml), aqueous KHSO<sub>4</sub> (0.5 M, 10 ml), sat. aq. NaHCO<sub>3</sub> (10

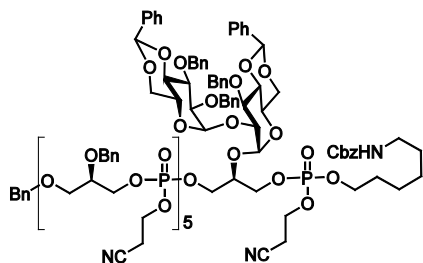
ml) and brine (10 ml). The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*, after which the residue was purified by size-exclusion chromatography (sephadex LH-20, THF), followed by column chromatography (MeOH/DCM), yielding dimethoxytritylated intermediate **25** (243 mg, 88.6 μmol, 86%) as white foam. <sup>31</sup>P NMR (161.7 MHz): δ = -1.3 - -0.9; <sup>1</sup>H NMR (400 MHz): δ = 2.29 - 2.56 (m, 10H, 5 x CH<sub>2</sub> cyanoethyl), 3.10 - 4.36 (m, 58H, 5 x CH<sub>2</sub> cyanoethyl, 6 x CH glycerol, 12 x CH<sub>2</sub> glycerol, 2 x OMe, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 4 x H-6), 4.45 - 4.89 (m, 18H, 9 x CH<sub>2</sub> Bn), 4.92 - 4.94 (m, 1H, H-1), 5.10 - 5.13 (m, 1H, H-1), 5.52 (s, 1H, CH benzylidene), 5.56 (s, 1H, CH benzylidene), 6.74 (d, 4H, *J* = 8.6 Hz, H<sub>arom</sub>), 7.14 - 7.48 (m, 64H, H<sub>arom</sub>); HRMS: [C<sub>143</sub>H<sub>158</sub>N<sub>5</sub>O<sub>40</sub>P<sub>5</sub> + Na]<sup>2+</sup> requires 1392.9478, found 1392.9489.



### Hexamer (26)

Intermediate **25** (122 mg, 44.5 μmol) was dissolved in a mixture of DCM (2.5 ml) and MeOH (25 ml). After the addition of pyridinium *para*-toluenesulphonate (PPTS, 25 mg, 0.099 mmol), the solution was allowed to stir overnight. Toluene (50 ml) was added and the mixture was concentrated partially (leaving ~30 ml) under reduced pressure. Column chromatography of the solution (MeOH/DCM) yielded hexamer **26** (76.0 mg, 31.1 μmol, 70%) as colourless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.3 - -0.8; <sup>1</sup>H NMR (400 MHz): δ = 1.79 (bs, 1H, OH),

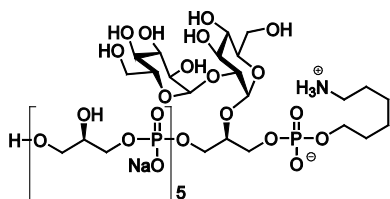
2.44 - 2.57 (m, 10H, 5 x CH<sub>2</sub> cyanoethyl), 3.38 - 4.34 (m, 52H, 5 x CH<sub>2</sub> cyanoethyl, 6 x CH glycerol, 12 x CH<sub>2</sub> glycerol, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 4 x H-6), 4.51 - 4.65 (m, 12H, 6 x CH<sub>2</sub> Bn), 4.76 - 4.99 (m, 7H, 3 x CH<sub>2</sub> Bn, H-1), 5.11 - 5.13 (m, 1H, H-1), 5.50 (s, 1H, CH benzylidene), 5.55 (s, 1H, CH benzylidene), 7.15 - 7.17 (m, 3H, H<sub>arom</sub>), 7.26 - 7.40 (m, 48H, H<sub>arom</sub>), 7.42 - 7.47 (m, 4H, H<sub>arom</sub>); HRMS: [C<sub>122</sub>H<sub>140</sub>N<sub>5</sub>O<sub>38</sub>P<sub>5</sub> + Na]<sup>2+</sup> requires 1241.8824, found 1241.8837.



### Hexamer-spacer (27)

To a solution of kojibiose substituted hexamer **26** (124 mg, 51.0 μmol) and Z-aminoethylphosphoramidite **5** (184 mg, 0.408 mmol) in acetonitrile (3.0 ml), containing freshly activated MS3Å, was added DCI (0.25 M in acetonitrile, 2.0 ml). After stirring the mixture for 4 h, acetonitrile (5.0 ml), I<sub>2</sub> (0.2 M in THF/pyridine 4/1, 3.1 ml) and H<sub>2</sub>O (1.0 ml) were added and the mixture stirred for 1 h. The mixture was diluted

with EtOAc (30 ml) and subsequently washed with sat. aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (10 ml), aqueous  $\text{KHSO}_4$  (0.5 M, 10 ml), sat. aq.  $\text{NaHCO}_3$  (10 ml) and brine (10 ml). The organic layer was dried with  $\text{Na}_2\text{SO}_4$  and concentrated *in vacuo*, after which the residue was purified by size-exclusion chromatography (sephadex LH-20, MeOH/DCM), followed by column chromatography (MeOH/DCM), yielding compound **27** (111 mg, 39.5  $\mu\text{mol}$ , 77%) as colourless oil.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta = -1.3$  -  $-0.9$ ;  $^1\text{H}$  NMR (400 MHz):  $\delta = 1.18$  -  $1.32$  (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.35 - 1.44 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.53 - 1.62 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.44 - 2.58 (m, 12H, 6 x  $\text{CH}_2$  cyanoethyl), 3.05 - 3.13 (m, 2H,  $\text{CH}_2$ -N hexylspacer), 3.56 - 4.33 (m, 56H, 6 x  $\text{CH}_2$  cyanoethyl, 6 x CH glycerol, 12 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$ -O hexylspacer, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 4 x H-6), 4.50 - 4.59 (m, 12H, 6 x  $\text{CH}_2$  Bn), 4.74 - 5.15 (m, 10H, 4 x  $\text{CH}_2$  Bn, 2 x H-1), 5.53 (s, 1H, CH benzylidene), 5.55 (s, 1H, CH benzylidene), 7.16 - 7.18 (m, 3H,  $\text{H}_{\text{arom}}$ ), 7.26 - 7.39 (m, 53H,  $\text{H}_{\text{arom}}$ ), 7.43 - 7.48 (m, 4H,  $\text{H}_{\text{arom}}$ ); HRMS:  $[\text{C}_{139}\text{H}_{163}\text{N}_7\text{O}_{43}\text{P}_6 + \text{Na}]^{2+}$  requires 1424.9497 found 1424.9512.



### Teichoic acid (**1**)

A solution of protected hexamer **27** (51.0 mg, 18.2  $\mu\text{mol}$ ) in dioxane (5.0 ml) was diluted with ammonia (25% in  $\text{H}_2\text{O}$ , 5.0 ml) and stirred overnight. The solution was concentrated *in vacuo* and the residue redissolved in  $\text{H}_2\text{O}$  (2.0 ml). The solution was then eluted through a small column containing Amberlite  $\text{Na}^+$  resin. The volatiles were removed under reduced pressure and the residue lyophilized, yielding the partially protected intermediate (47.6 mg, 100%) as amorphous white solid. A part of the intermediate (23.6 mg, 9.01  $\mu\text{mol}$ ) was dissolved in a mixture of dioxane (2.0 ml),  $\text{H}_2\text{O}$  (2.0 ml) and AcOH (three drops). After the addition of Palladium black (~50 mg, 0.5 mmol), the mixture was allowed to stir for 5 days under a hydrogen atmosphere. The mixture was filtered, concentrated *in vacuo*, and purified by size exclusion chromatography (Sephadex HW40, 0.15M  $\text{NH}_4\text{HCO}_3$ ). The purified residue was lyophilized twice before it was dissolved in  $\text{H}_2\text{O}$  (2.0 ml) and eluted through a small column containing Amberlite  $\text{Na}^+$  resin. Cryodesiccation of the filtrate yielded deprotected teichoic acid **1** (10.6 mg, 6.81  $\mu\text{mol}$ , 76%) as amorphous white solid.  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 0.9$  (1P), 1.1 (1P), 1.2 (3P), 1.3 (1P);  $^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.36$  -  $1.40$  (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.59 - 1.66 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.95 (t, 2H,  $J = 7.5$  Hz,  $\text{CH}_2$ -N hexylspacer), 3.37 - 3.41 (m, 2H, 2 x H-5), 3.51 - 3.56 (m, 2H, CHH glycerol, H-2), 3.61 - 3.64 (m, 2H, CHH glycerol, H-2), 3.70 - 4.01 (m, 37H, 5 x CH glycerol, 11 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$ -O hexylspacer, 2 x H-3, 2 x H-4, 4 x H-6), 4.11 - 4.14 (m, 1H, CH glycerol), 5.11 (d, 1H,  $J = 3.7$  Hz, H-1), 5.39 (d, 1H,  $J = 3.5$  Hz, H-1);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 25.4$ , 26.1, 27.6 (3 x  $\text{CH}_2$  hexylspacer), 30.4 (d,  $J = 6.7$  Hz,  $\text{CH}_2$  hexylspacer), 40.4 ( $\text{CH}_2$ -N hexylspacer), 61.4, 61.4 (2 x C-6), 63.0 ( $\text{CH}_2$  glycerol), 65.5 (d,  $J = 5.1$  Hz,  $\text{CH}_2$  glycerol), 66.0 (d,  $J = 4.4$  Hz,  $\text{CH}_2$  glycerol), 67.0 - 67.3 (9 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$ -O hexylspacer), 70.4 - 70.6 (4 x CH glycerol, 2 x C-5), 71.7 (d,  $J = 7.8$  Hz, CH glycerol), 72.1 (C-2, C-3), 72.7 (2 x C-4), 73.3 (C-3), 75.1 (C-2), 76.0 (t,  $J = 8.3$  Hz, CH glycerol), 95.6 (C-1), 96.4 (C-1). HRMS:  $\text{C}_{36}\text{H}_{77}\text{NO}_{41}\text{P}_6 + \text{H}^+$  requires 1366.2469 found 1366.2483.

## References

1. Fischer, W. *Adv. Microb. Physiol.* **1988**, 29, 233-302.
2. Neuhaus, F.C.; Baddiley, J. *Microbiol. Mol. Biol. Rev.* **2003**, 67, 686-723.
3. Weidenmaier, C.; Peschel, A. *Nat. Rev. Microbiol.* **2008**, 6, 276-287.
4. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Sava, I.; Ye, L.; Bychowska, A.; Holst, O.; Huebner, J. *Plos One* **2011**, 6, e17839.
5. Theilacker, C.; Kropec, A.; Hammer, F.; Sava, I.; Wobser, D.; Sakinc, T.; Codée, J.D.C.; Hogendorf, W.F.J.; Van der Marel, G.A.; Huebner, J. *J. Infect. Dis.* **2012**, 205, 1076-1085.
6. Morath, S.; Stadelmaier, A.; Geyer, A.; Schmidt, R. R.; Hartung, T. *J. Exp. Med.* **2002**, 195, 1635-1640.
7. Stadelmaier, A.; Morath, S.; Hartung, T.; Schmidt, R.R. *Angew. Chem., Int. Ed.* **2003**, 42, 916-920.
8. Deininger, S.; Stadelmaier, A.; von Aulock, S.; Morath, S.; Schmidt, R.R.; Hartung, T. *J. Immun.* **2003**, 170, 4134-4138.
9. Morath, S.; von Aulock, S.; Hartung, T. *J. Endotoxin Res.* **2005**, 6, 348-356.
10. Figueroa-Perez, I.; Stadelmaier, A.; Morath, S.; Hartung, T.; Schmidt, R.R. *Tetrahedron: Asymmetry* **2005**, 16, 493-506.
11. Figueroa-Perez, I.; Stadelmaier, A.; Deininger, S.; von Aulock, S.; Hartung, T.; Schmidt, R.R. *Carbohydr. Res.* **2006**, 341, 2901-2911.
12. Stadelmaier, A.; Figueroa-Perez, I.; Deininger, S.; von Aulock, S.; Hartung, T.; Schmidt, R.R. *Bioorg. Med. Chem.* **2006**, 14, 6239-6254.
13. Koch, S.; Hufnagel M.; Huebner, J. *Expert Opin. Biol. Ther.* **2004**, 4, 1519-1531.
14. Theilacker, C.; Krueger, W.A.; Kropec, A.; Huebner, J. *Vaccine* **2004**, 22, S31-S38.
15. Hidron, A.I.; Edwards, J.R.; Patel, J.; Horan, T.C.; Sievert, D.M.; Pollock, D.A.; Fridkin, S.K. *Infect. Control Hosp. Epidemiol.* **2008**, 29, 996-1011.
16. Huebner, J.; Wang, Y.; Krueger, W.A.; Madoff, L.C.; Martirosian, G.; Boisot, S.; Goldmann, D.A.; Kasper, D.L.; Tzianabos, A.O.; Pier, G. B. *Infect. Immun.* **1999**, 67, 1213-1219.
17. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Fabretti, F.; Sange, T.; Holst, O.; Huebner, J. *Infect. Immun.* **2006**, 74, 5703-5712.
18. Hogendorf, W.F.J.; Van den Bos, L.J.; Overkleef, H.S.; Codée, J.D.C.; van der Marel, G.A. *Bioorg. Med. Chem.* **2010**, 18, 3668-3678.
19. Caruthers, M.H. *Science* **1985**, 230, 281-285.
20. Beaucage, S.L.; Iyer, R.P. *Tetrahedron* **1992**, 48, 2223-2311.
21. Reese, C.B. *Org. Biomol. Chem.* **2005**, 3, 3851-3868.
22. Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H.G. *J. Am. Chem. Soc.* **1963**, 85, 3821-3827.
23. Eibl, H. *Chem. Phys. Lip.* **1981**, 28, 1-5.
24. van Boeckel, C.A.A.; Visser, G.M.; van Boom, J.H. *Tetrahedron* **1985**, 41, 4557-4565.
25. Oltvoort, J.J.; van Boeckel, C.A.A.; De koning, J.H.; van Boom, J.H. *Synthesis* **1981**, 305-308.
26. Kim, J.-H.; Yang, H.; Park, J.; Boons, G.-J. *J. Am. Chem. Soc.* **2005**, 127, 12090-12097.
27. Kim, J.-H.; Yang, H.; Boons, G.-J. *Angew. Chem., Int. Ed.* **2005**, 44, 947-949.
28. Kim, J.-H.; Yang, H.; Khot, V.; Whitfield, D.; Boons, G.-J. *Eur. J. Org. Chem.* **2006**, 5007-5028.
29. Lemieux, R.U.; Hendriks, K.B.; Stick, R.V.; James, K. *J. Am. Chem. Soc.* **1975**, 97, 4056-4062.
30. van Boeckel, C.A.A.; van Boom, J.H. *Chem. Lett.* **1981**, 581-584.
31. Adinolfi, M.; Barone, G.; Iadonisi, A.; Shiattarella, M. *Tetrahedron Lett.* **2002**, 43, 5573-5577.
32. Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, 44, 6479-6482.
33. Walvoort, M.T.C.; Lodder, G.; Overkleef, H.S.; Codée, J.D.C.; Van der Marel, G.A. *J. Org. Chem.* **2010**, 75, 7990-8002.

34. In **chapter five**, the glycosylation of glycerol **7** using the 6-*O*-FMOc protected glucosyl imidate donor from reference 33 is described.
35. Crich, D.; de la Mora, M.; Vinod, A.U. *J. Org. Chem.* **2003**, *68*, 8142-8148.
36. Codée, J.D.C.; Stubba, B.; Schiattarella, M.; Overkleeft, H.S.; van Boeckel, C.A.A.; van Boom, J.H.; van der Marel, G.A. *J. Am. Chem. Soc.* **2005**, *127*, 3767-3773.
37. Codée, J.D.C.; Litjens, R.E.J.N.; den Heeten, R.; Overkleeft, H.S.; van Boom, J.H.; van der Marel, G.A. *Org. Lett.* **2003**, *5*, 1519-1522.
38. Fukase, K.; Hashida, M.; Kusumoto, S. *Tetrahedron Lett.* **1991**, *32*, 3557-3558.
39. Zheng, W.; DeMattei, J.A.; Wu, J.-P.; Duan, J.J.-W.; Cook, L.R.; Oinuma, H.; Kishi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 7946-7968.
40. Hogendorf, W.F.J.; Meeuwenoord, N.; Overkleeft, H.S.; Filippov, D.V.; Laverde, D.; Kropec, A.; Huebner, J.; van der Marel, G.A.; Codée, J.D.C. *Chem. Commun.* **2011**, *47*, 8961-8963.
41. Chipowsky, S.; Lee, Y.C. *Carbohydr. Res.* **1973**, *31*, 339-346.

## Chapter 3

---

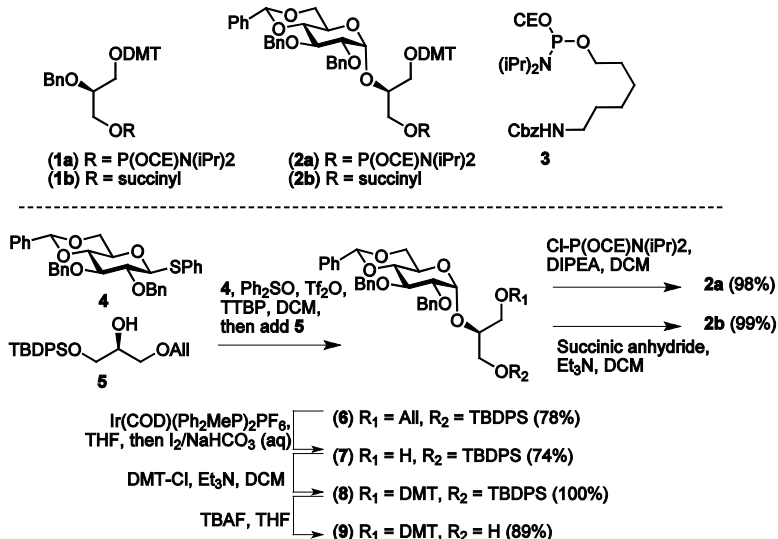
# Automated Solid Phase TA Synthesis

### Introduction

Teichoic acids (TAs) have been implied as antigenic structures<sup>1,2</sup> and are therefore interesting targets for the development of (synthetic) vaccines against increasingly resistant Gram-positive species, such as *E. faecalis*<sup>3,4</sup> and *S. aureus*.<sup>5,6</sup> Because of the microheterogeneity of the TA in biological preparations, the establishment of structure activity relations has been troublesome and the biological activity of TA preparations from biological sources has been subject of debate.<sup>7-9</sup> Therefore, a library of synthetic TA structures would be a powerful means to find the optimal length and substitution pattern of an antigen. In **chapter two** the solution phase synthesis of a (kojibiosyl) GTA hexamer is described.<sup>10</sup> Although this approach can be used for a large scale synthesis of a single target compound, it is too labor intensive to generate a library of compounds.

Automated solid phase synthesis is a well established methodology in oligonucleic acid synthesis, giving rapid access to (a library of) oligomers of desired length and composition.<sup>11-13</sup> Because of their repetitive nature and connection through phosphodiester linkages, TAs resemble oligonucleic acids. Therefore, an automated solid phase approach, in combination with the chemistry described in **chapter two**, was deemed suitable to obtain a set of synthetic TA fragments.

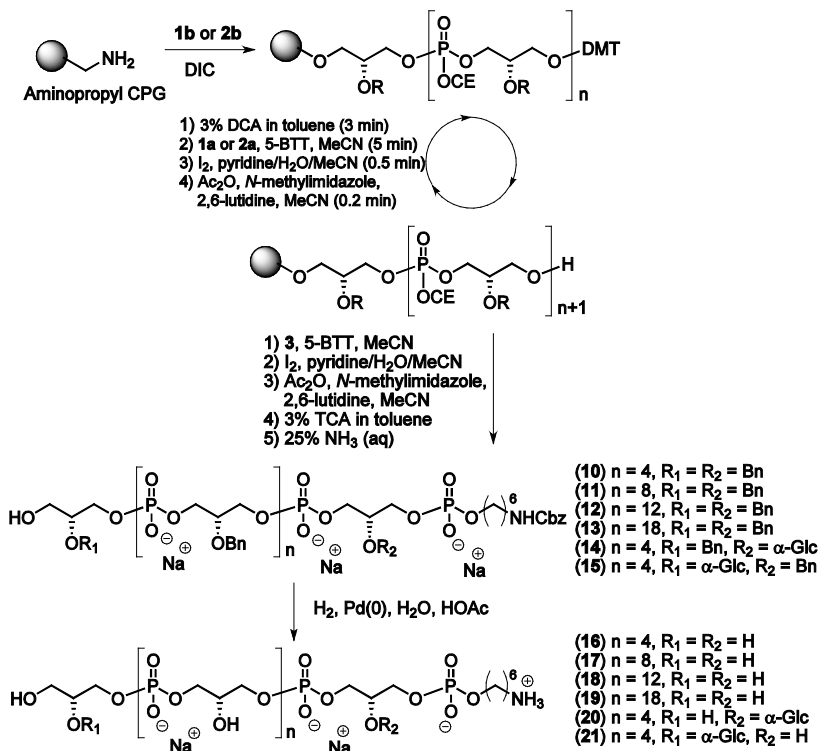
This chapter describes the synthesis of GTA oligomers up to a length of a 20-mer, using a commercial DNA synthesizer. Glucosylated fragments, a substitution that is found in several species of *Staphylococcus* and *Bacillus*,<sup>14,15</sup> have been obtained using tailor-made glucosyl glycerol phosphoramidite. The library of synthetic TAs has been evaluated on their antigenicity in an opsonophagocytic inhibition assay (OPIA), revealing a length-activity relationship and a high inhibitory potential of two glucosylated hexaglycerol phosphates.<sup>16</sup>

**Scheme 1.** Building blocks for the automated solid phase TA synthesis.

## Results and Discussion

Based on the state-of-the-art in nucleic acid synthesis, and as described in **chapter two**, cyanoethyl (CE) protected glycerol phosphoramidite building blocks were employed (see scheme 1).<sup>13</sup> The alcohol functions of the building blocks, that were used for elongation, mediated glycosylation of glycerol alcohol **5**.<sup>18-21</sup> The resulting glucosylglycerol **6** was transformed into **2a/b** as follows: iridium catalyzed isomerization of the allyl ether<sup>22</sup> and subsequent cleavage of the resulting enol ether using iodine under basic conditions liberated the primary alcohol (**7**), which was capped with a DMT group to give compound **8**. Next the silyl group was removed to provide alcohol **9**, which was either phosphitylated to provide the phosphoramidite building block **2a**, or treated with succinic anhydride to give the glucosylglycerol linker **2b**.

With the required building blocks in hand the next goal was to explore the automated solid phase TA oligomer synthesis (Scheme 2). As a first objective the assembly of spacer containing TA-hexamer **10** was investigated. To this end aminopropyl CPG was functionalized with succinyl glycerol **1b**. Cleavage of the DMT group with 3% dichloroacetic acid (DCA) in toluene and concomitant determination of the loading led to a CPG with a loading of 100  $\mu\text{mol/g}$ . At this point, the CPG-glycerol was installed into the ÄKTA™ oligopilot™ DNA/RNA synthesizer. For the coupling step the resin was treated with 5 equiv. of phosphoramidite **1a** and 5-benzylthiotetrazole<sup>23</sup> (5-BTT, 22.5 equiv.) as activator in acetonitrile for 5 minutes. Oxidation of the intermediate phosphites was achieved with I<sub>2</sub> in pyridine/H<sub>2</sub>O (1 min.), after which a

**Scheme 2.** Automated solid phase TA synthesis.

capping step (*N*-methylimidazole/acetic anhydride (Ac<sub>2</sub>O)/2,6-lutidine) was introduced to cap any unreacted alcohol functionalities. To complete the elongation cycle the DMT-groups were removed with 3% DCA in toluene. The coupling efficiency using this protocol was generally > 98% as judged from the automatic DMT-count. In the 6th cycle of the hexamer assembly, the glycerol phosphoramidite **1a** was replaced by benzyloxy-carbonyl aminohexanol phosphoramidite **3** to terminate the sequence.<sup>24</sup> Cleavage of the hexamer from the CPG using aqueous ammonia, and concomitant cyanoethyl removal liberated the partially protected TA-hexamer **10**. Two different protocols for the purification of the partially protected oligomers were examined: RP-HPLC purification and anion exchange chromatography followed by desalination. In the case of hexamer **10**, the former protocol provided the desired oligomer in the highest yield (18% overall from aminopropyl CPG).

There was a possibility that the phosphodiester linkages in the synthetic targets would be labile to base treatment, in analogy to the lability of RNA fragments.<sup>13</sup> To investigate the base lability of the partially protected TA oligomer, hexamer **10** was subjected to a treatment with 25% aqueous ammonia for a prolonged period of time at room temperature and at 40 °C. No degradation of the hexamer could be detected by LCMS analysis after 24h at 40 °C, indicating that transesterification of the

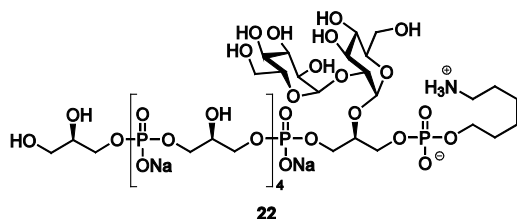


phosphodiester is not a significant risk under the reaction conditions used. Using the conditions described above, the following TA-oligomers were assembled: unsubstituted 10-mer **11**, unsubstituted 14-mer **12**, unsubstituted 20-mer **13** and mono glucosyl substituted 6-mers **14** and **15**. TA-fragment **15** was assembled on CPG resin functionalized with glucosyl glycerol succinate **2b** (loading 100  $\mu\text{mol/g}$ ). The results of the syntheses are summarized in Table 1, from which it becomes clear that the syntheses of the longer and substituted fragments proceeded with even greater efficiency than the assembly of hexamer **10**. With increasing size of the oligomers, purification by ion exchange chromatography became more efficient than RP-HPLC purification. For TA 20-mer **13** and the glucosyl substituted hexamers **14** and **15** LCMS analysis of the crude reaction products showed significant peak broadening and therefore these compounds were solely purified using the ion-exchange protocol. From the excellent yields of glucosyl substituted hexamers **14** and **15** it becomes clear that the glycerol C-2 glucose substitution had no adverse effect on the coupling efficiency of the building blocks used. Notably, the benzylidene functionality on the glucosyl moiety in **15** proved to be stable to the repeated detritylation steps. Global deprotection of the partially protected TA-fragments was achieved by hydrogenolysis of the benzyl ethers, Cbz-group and benzylidene functionality over palladium black followed by desalination (Scheme 2), uneventfully leading to TA-target structures **16-21**, as summarized in Table 1. The structure of the final products was confirmed by NMR spectroscopy and high resolution mass spectrometry. In the NMR spectra the relative ratio of the integrals of the peaks belonging to the spacer  $\text{CH}_2$  groups, the spacer  $\text{CH}_2\text{-N}$  group and the terminal glycerol  $\text{CH}_2\text{-O}$  moiety with respect to the bulk signal originating from all other glycerol protons, provided proof for the integrity of the structures.

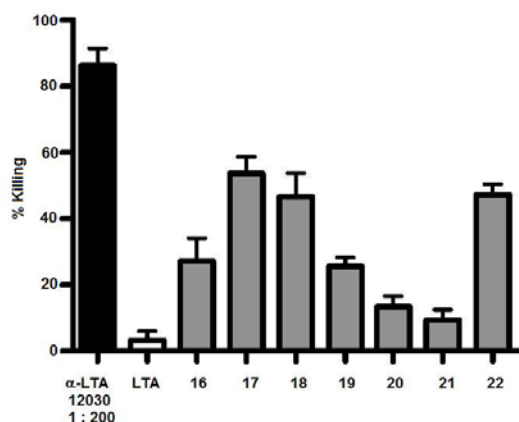
To establish their antigenic activity, the prepared TA fragments (**16-21**) were tested in an opsonophagocytic inhibition assay, as described by Theilacker *et al.*<sup>3,4,16</sup> Also, kojibiosyl GTA hexamer **22** (figure 1), of which the synthesis is described in **chapter two** was taken along in this biological evaluation. In this assay, rabbit serum raised against purified *E. faecalis* LTA is used to kill *E. faecalis* bacteria. Blocking the

**Table 1.** Automated solid phase synthesis and deprotection yields.

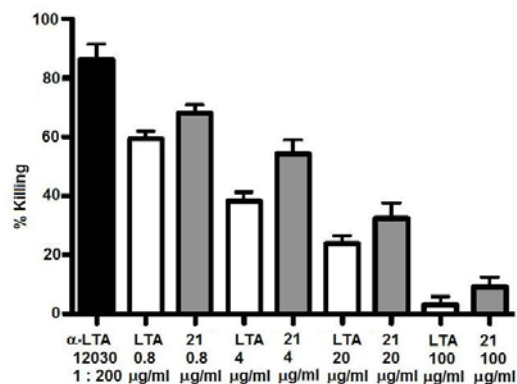
Entry	Compound	Yield (RP-HPLC)	Yield (ion exchange)	Deprotection (yield)
1	<b>10</b>	18%	10%	<b>16</b> (65%)
2	<b>11</b>	29%	16%	<b>17</b> (78%)
3	<b>12</b>	8%	21%	<b>18</b> (84%)
4	<b>13</b>		24%	<b>19</b> (95%)
5	<b>14</b>		32%	<b>20</b> (68%)
6	<b>15</b>		36%	<b>21</b> (86%)



**Figure 1.** Hexamer 22.



**Figure 2.** Results OPIA. α-LTA 12030 represents the killing of the serum at 1:200 dilution. LTA is used as a positive control. Compounds **16-22** are used in a concentration of 100 µg/ml.



**Figure 3.** OPIA of LTA and compound **21** at different concentrations.

opsonic antibodies in the serum with an inhibitor will lead to reduced killing. The inhibitory potential of the synthesized TA fragments at a concentration of 100 µg/mL is displayed in Figure 2, which shows that the smallest fragment tested, hexamer **16**, is capable of inhibiting the opsonophagocytic killing. For the 10-, 14- and 20-mer a length-dependence is observed for the inhibitory potential. The longer the fragments are the better the binding is to the opsonic antibodies, resulting in reduced killing. Interestingly, the glucose substituted TA fragments **20** and **21** were found to be relatively very potent inhibitors. This is striking since the α-glucosyl substituent is known from the TA from several *Staphylococcus* and *Bacillus* strains, but has not been found in *E. faecalis* TA.<sup>14,15</sup> In contrast, hexamer **22**, bearing the kojibiosyl substitution naturally occurring in *E. faecalis* TA, showed a significantly lower inhibition. Compound **21** proved to be the most active compound of the series, showing already some inhibition at a concentration of 0.8 µg/ml. Furthermore, compound **21** showed a comparable concentration-inhibition relation as native LTA (see figure 3), which makes this a promising candidate for the future development of a vaccine comprising **21** or a close analogue as a synthetic TA-antigen.<sup>16</sup>

## Conclusion

This chapter describes the development of automated solid phase methodology to synthesize glycerol phosphate teichoic acid fragments. Tailor made glycerol phosphoramidite building blocks were used as building blocks in combination with a commercially available synthesizer, to produce partially protected TA fragments. With a full coupling cycle, taking approximately 15 minutes, a TA 20-mer was produced in 5 hours. Functionalized glycerol phosphate building blocks could also be used in the synthesis to allow the assembly of substituted TA fragments. Employing the solid phase methodology, a small library of TA fragments was generated which was tested for activity in an opsonophagocytic inhibition assay, revealing a clear TA-length-activity relationship. The assay also revealed glucosyl substituted TA-hexamers **20** and **21** as promising lead candidates for future vaccine development. Interestingly, hexamer **22**, containing the kojibiosyl substitution as found in *E. Faecalis* LTA, did not show any opsonophagocytic inhibition. Hexamer **21** can also be synthesized in a light fluoruous fashion (see **chapter five**) and conjugated to several immunogenic carrier proteins (see **chapter seven**).

## Experimental section

**General Procedures and Material:** All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma-Aldrich, Genscript) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H<sub>2</sub>SO<sub>4</sub> in ethanol or with a solution of (NH<sub>4</sub>)<sub>4</sub>Ce(SO<sub>4</sub>)<sub>4</sub>·2H<sub>2</sub>O 10 g/l, in 10% aqueous H<sub>2</sub>SO<sub>4</sub> followed by charring at +/- 140 °C. Some unsaturated compounds were visualized by spraying with a solution of KMnO<sub>4</sub> (2%) and K<sub>2</sub>CO<sub>3</sub> (1%) in H<sub>2</sub>O. Optical rotation measurements ([ $\alpha$ ]<sub>D</sub><sup>20</sup>) were performed on a Propol automated polarimeter (Sodium D-line,  $\lambda$  = 589 nm) with a concentration of 10 mg/ml (c = 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300 and are reported in cm<sup>-1</sup>. <sup>31</sup>P, <sup>1</sup>H, and <sup>13</sup>C NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 100 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in CDCl<sub>3</sub> with chemical shift ( $\delta$ ) relative to tetramethylsilane, unless stated otherwise. High resolution mass spectra were recorded by direct injection (2  $\mu$ l of a 2  $\mu$ M solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). LC-MS analysis was conducted on a JASCO system using an Alltima C18 analytical column (5  $\mu$ m particle size, flow: 1.0 ml/min). Absorbance was measured at 214 nm. Solvent system: A: water, B: MeCN, C: 0.15 M NH<sub>4</sub>OAc in H<sub>2</sub>O, gradient (13.5 min): 80% A, 10% B, 10% C  $\rightarrow$  0% A, 90% B, 10% C. This system was coupled to a PE/SCIEX API165 (Perkin-Elmer) mass spectrometer.

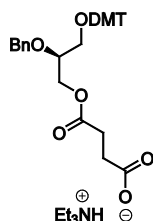
**Procedure for automated solid-phase synthesis, purification and global deprotection of TA oligomers:** Aminopropyl modified controlled pore glass support (CPG, Fluka) was loaded with (glucosyl)glycerol succinates **1b** or **2b** and the loading was determined (loading: 100  $\mu\text{mol/g}$  CPG) using the method described by Pon.<sup>25</sup> The automated syntheses were performed on a synthesizer (ÄKTA™ oligopilot plus™, GE Healthcare) on a scale of 100-150 mg functionalized CPG (10-15  $\mu\text{mol}$  glycerol derivative) and started off with acidolysis of the dimethoxytrityl ether using 3% dichloroacetic acid in toluene (15 ml, 3 min). After flushing with acetonitrile (5ml, 1 min), the resulting alcohol was reacted with phosphoramidites **1a** or **2a** (0.1 M in MeCN, 5 eq) and 5-benzylthiotetrazole (BTT, 0.3M in MeCN, 22.5 eq) for 5 min using a cycled flow. After flushing with MeCN (5ml, 1 min), oxidation of the intermediate phosphite was performed using  $\text{I}_2$  (0.05 M in pyridine/ $\text{H}_2\text{O}$  9/1, 2ml, 1 min). A flushing step with MeCN (5ml, 1 min) was followed by a capping step (1 ml of a 1/1 mixture of capping solution A (20 v/v% *N*-methylimidazole in MeCN) and capping solution B (20 v/v%  $\text{Ac}_2\text{O}$ , 15 v/v% 2,6-lutidine in MeCN for 12s). After flushing with MeCN (5ml, 1 min), a detritylation step was performed using the before mentioned cocktail and the molecule was elongated using phosphoramidites **1a** or **2a** using the same set of reactions (coupling, oxidation, capping, detritylation). The average coupling efficiency was measured by quantitative UV-detection (400 nm) of the dimethoxytrityl cation during each detritylation step. When the desired length was obtained, spacer phosphoramidite **3** (0.1 M in MeCN, 2 x 5 eq, 2 x 5 min) was coupled to the CPG-TA-oligomer using 5-BTT (0.3M in MeCN, 2 x 22.5 eq) and, subsequently treated with  $\text{I}_2$  (0.05 M in pyridine/ $\text{H}_2\text{O}$  9/1, 2ml, 1 min), before it was released from the solid support using 25%  $\text{NH}_4\text{OH}$  (10ml, 1h, the cyanoethyl groups are concomitantly released at this stage). The solvents were then removed *in vacuo* before the crude oligomer was purified using method A or B.

**Purification method A:** RP-HPLC (Gilson preparative HPLC system; column: Alltima C18, particle size: 5  $\mu\text{m}$ , dimensions: 10/250 mm; eluent: (10 mM  $\text{NH}_4\text{OAc}$  in  $\text{H}_2\text{O}$ )/MeCN, 9/1  $\rightarrow$  1/9, detection: UV (215 and 254 nm), the fractions containing product were collected and the solvents were removed under reduced pressure. Repeated lyophilization (twice) of the residue was followed by eluting the purified oligomer through a small column containing Dowex  $\text{Na}^+$  cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in  $\text{H}_2\text{O}$ , flushed with  $\text{H}_2\text{O}$  and MeOH before use). Lyophilization gave the partially protected oligomer of which the integrity and purity was confirmed by LC-MS, HRMS and NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ ) analysis.

**Purification method B:** Anion-exchange chromatography (device: ÄKTA Explorer™, GE Healthcare; column: Q-sepharose HR16/10, GE Healthcare; eluent: buffer A (50 mM NaOAc, 50 mM  $\text{NaClO}_4$ ), buffer B (50mM NaOAc, 500mM  $\text{NaClO}_4$ ), gradient 1/0  $\rightarrow$  0/1) followed by desalination using size-exclusion chromatography (Sephadex G10 (hexamer **10**) or Sephadex G25 (all other oligomers), GE Healthcare, dimensions: 26/60 mm, eluent: 0.15 M  $\text{NH}_4\text{HCO}_3$ ). The purified oligomer was lyophilized twice before it was eluted through a small column containing Dowex  $\text{Na}^+$  cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in  $\text{H}_2\text{O}$ , flushed with  $\text{H}_2\text{O}$  and MeOH before use). Lyophilization gave the partially protected oligomer of which the integrity and purity was confirmed by LC-MS, HRMS and NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ ) analysis.

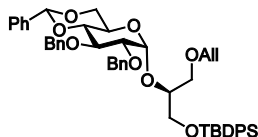
**Deprotection:** The oligomers (1-5  $\mu\text{mol}$ ) were dissolved in  $\text{H}_2\text{O}$  (3-6 ml) together with AcOH (3-6 drops) and treated for 3 days with Palladium black (20-40 mg)/ $\text{H}_2$ . Subsequently, the mixture was filtered and the solvents removed under reduced pressure before the residue was purified by size-exclusion chromatography (Sephadex HW40, Toyopearl, dimensions: 16/60 mm, eluent: 0.15 M  $\text{Et}_3\text{NHOAc}$  or 0.15 M  $\text{NH}_4\text{OAc}$ ). After repeated lyophilization, the purified product was eluted through a small column containing Dowex  $\text{Na}^+$  cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in  $\text{H}_2\text{O}$ , flushed with  $\text{H}_2\text{O}$  and MeOH before use).

Lyophilization gave the partially protected oligomer of which the integrity and purity was confirmed by HRMS and NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ ) analysis.



**1-O-(Triethylammonium succinate)-2-O-benzyl-3-O-(4,4'-dimethoxytrityl)-sn-glycerol (**1b**)**

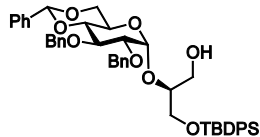
To a solution of 2-O-benzyl-3-O-(4,4'-dimethoxytrityl)-sn-glycerol (2.50 g, 5.16 mmol) and  $\text{Et}_3\text{N}$  (7.87 ml, 56.8 mmol) in DCM (35 ml) was added succinic anhydride (2.58 g, 25.8 mmol). The mixture was stirred for 10 min at 0 °C, followed by the addition of a catalytic amount of DMAP. After stirring 2h at RT, the mixture was diluted with DCM (80 ml) and washed with 0.5 M HCl (50 ml), sat. aq.  $\text{NaHCO}_3$  (40 ml) and brine (40 ml). The organic layer was dried over  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. Purification of the residue by column chromatography ( $\text{MeOH}/\text{DCM}/\text{Et}_3\text{N}$ ), afforded glycerol succinate **1b** (3.26 g, 4.75 mmol, 92%) as white foam.  $[\alpha]_{\text{D}}^{20}$  ( $\text{MeOH}$ ): +8.2; IR: 829, 1034, 1177, 1250, 1609, 1736, 2343, 2359;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{CN}$ ):  $\delta$  = 1.11 (t, 9H,  $J$  = 7.3 Hz, 3 x  $\text{CH}_3$   $\text{Et}_3\text{N}$ ), 2.35 - 2.45 (m, 4H, 2 x  $\text{CH}_2$  succinyl), 2.85 (q, 6H,  $J$  = 7.3 Hz, 14.6 Hz, 3 x  $\text{CH}_2$   $\text{Et}_3\text{N}$ ), 3.14 - 3.22 (m, 2H, 2 x CHH glycerol), 3.72 - 3.74 (m, 7H, 2 x OMe, CH glycerol), 4.15 - 4.23 (m, 2H, 2 x CHH glycerol), 4.57 (s, 2H,  $\text{CH}_2$  Bn), 6.83 (d, 4H,  $J$  = 8.8 Hz,  $\text{H}_{\text{arom}}$ ), 7.17 - 7.33 (m, 12H,  $\text{H}_{\text{arom}}$ ), 7.44 (d, 2H,  $J$  = 7.6 Hz,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{CN}$ ):  $\delta$  = 9.2 (3 x  $\text{CH}_3$   $\text{Et}_3\text{N}$ ), 30.9 ( $\text{CH}_2$  succinyl), 31.7 ( $\text{CH}_2$  succinyl), 45.9 (3 x  $\text{CH}_2$   $\text{Et}_3\text{N}$ ), 55.8 (2 x OMe), 63.7 ( $\text{CH}_2$  glycerol), 64.4 ( $\text{CH}_2$  glycerol), 72.6 ( $\text{CH}_2$  Bn), 77.2 (CH glycerol), 86.8 ( $\text{C}_q$  DMT), 113.9 ( $\text{CH}_{\text{arom}}$ ), 127.6, 128.4, 128.6, 128.7, 128.8, 129.1, 130.8 ( $\text{CH}_{\text{arom}}$ ), 136.9, 139.7, 146.1, 159.5 ( $\text{C}_q$  Bn, 4 x  $\text{C}_q$  DMT), 173.8, 176.9 (2 x CO succinate); HRMS (free acid):  $\text{C}_{35}\text{H}_{36}\text{O}_8$  +  $\text{Na}^+$  requires 607.2302, found 607.2298.



**1-O-(tert-Butyldiphenylsilyl)-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene- $\alpha$ -D-glucopyranosyl)-3-O-allyl-sn-glycerol (**6**)**

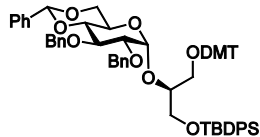
A solution of glucose donor **4** (2.70 g, 5.00 mmol), TTBP (2.86 g, 11.5 mmol) and  $\text{Ph}_2\text{SO}$  (1.11 g, 5.50 mmol) in freshly distilled DCM (100 ml), together with activated MS  $3\text{\AA}$ , was stirred under argon at RT for 30 min. The mixture was then cooled to -75 °C and stirred for another 15 min. After the addition of  $\text{Tf}_2\text{O}$  (0.93 ml, 5.5 mmol) the mixture was stirred for 45 min at -75 °C and, subsequently, glycerol acceptor **5** (2.22 g, 5.99 mmol) was added. After stirring for 2 hrs at -75 °C, the mixture was allowed to warm to room temperature overnight. The reaction was by the addition of moist  $\text{Et}_3\text{N}$  (3.4 ml, 25 mmol) and stirred for 30 min. After washing with sat. aq.  $\text{NaHCO}_3$  (30 ml) and brine (30 ml), the organic layer was dried over  $\text{MgSO}_4$  and concentrated *in vacuo*. The resulting oil was dissolved in pyridine (50 ml) and  $\text{Ac}_2\text{O}$  (10 ml) and stirred for 2 hrs. The solvents were removed *in vacuo* before the residue was redissolved in  $\text{Et}_2\text{O}$  (125 ml) and washed with  $\text{H}_2\text{O}$  (2 x 40 ml) and brine (40 ml). The organic layer was dried over  $\text{MgSO}_4$  and the solvent removed under reduced pressure. Column chromatography ( $\text{EtOAc}/\text{PE}$ ) of the residue gave  $\alpha$ -glucoside **6** (3.10 g, 3.87 mmol, 78%), as a colourless oil with a minor amount (< 7%, based on  $^1\text{H}$ -NMR analysis) of the  $\beta$ -product.  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): +4.2; IR: 737, 1088, 1369, 1454, 1751, 2855, 2924;  $^1\text{H}$  NMR (400 MHz,  $\alpha$ -anomer):  $\delta$  = 1.05 (s, 9H, *t*-Bu TBDPS), 3.53 - 3.69 (m, 4H, CH glycerol, CHH glycerol, H-2, H-6), 3.71 - 3.81 (m, 3H, 3 x CHH glycerol), 3.86 - 3.92 (m, 1H, H-5), 3.99 - 4.04 (m, 5H,  $\text{CH}_2$  allyl, H-3, H-4, H-6), 4.74 (d, 1H,  $J$  = 12.0 Hz, CHH Bn), 4.78 (d, 1H,  $J$  = 11.8 Hz, CHH Bn), 4.80 (d, 1H,  $J$  = 11.2 Hz, CHH Bn), 4.87 (d, 1H,  $J$  = 11.3 Hz, CHH Bn), 5.16 (dd, 1H,  $J$  = 1.3 Hz, 10.4 Hz, CHH allyl), 5.26 (dd, 1H,  $J$  = 1.6 Hz, 17.2 Hz, CHH allyl), 5.26 (d, 1H,  $J$  = 3.8 Hz, H-1), 5.50 (s, 1H, CH benzylidene), 5.88 (ddd, 1H,  $J$  = 5.2 Hz, 10.4 Hz, 22.4 Hz, CH allyl), 7.24 - 7.46 (m, 21H,  $\text{H}_{\text{arom}}$ ), 7.65 - 7.68 (m, 4H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz,  $\alpha$ -anomer):  $\delta$  = 19.2 ( $\text{C}_q$  *t*-Bu), 26.8 (3 x  $\text{CH}_3$  TBDPS), 62.4 (C-5), 63.9 ( $\text{CH}_2$  glycerol), 68.8 (C-6), 70.8 ( $\text{CH}_2$  glycerol), 72.3 ( $\text{CH}_2$  allyl), 72.5 ( $\text{CH}_2$  Bn), 75.2 ( $\text{CH}_2$  Bn), 76.7, 78.2 (C-3, C-

4), 79.0 (CH glycerol), 82.1 (C-2), 97.2 (C-1), 101.2 (CH benzylidene), 116.9 (CH<sub>2</sub> allyl), 126.0 - 129.7 (CH<sub>arom</sub>), 133.1, 133.2 (2 x C<sub>q</sub> phenyl), 134.6 (CH allyl), 135.5 (CH<sub>arom</sub>), 137.5, 138.3, 138.8 (2 x C<sub>q</sub> Bn, C<sub>q</sub> benzylidene); HRMS: C<sub>49</sub>H<sub>56</sub>O<sub>8</sub>Si + Na<sup>+</sup> requires 823.3637, found 823.3631.



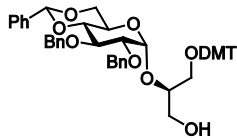
**1-O-(tert-Butyldiphenylsilyl)-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-sn-glycerol (7)**

A solution of glycoside **6** (0.400 g, 0.499 mmol) in freshly distilled THF (3.0 ml) was stirred under argon for 30 min. After the addition of Ir(COD)(Ph<sub>2</sub>MeP)<sub>2</sub>PF<sub>6</sub> (0.042 g, 10 mol%) the solution turned red and the mixture was purged with H<sub>2</sub> (g) until the solution turned colourless again (~30s). After stirring under argon for 2 h, the mixture was diluted with THF (7.0 ml) and sat. aq. NaHCO<sub>3</sub> (25 ml). Upon addition of I<sub>2</sub> (0.190 g, 0.75 mmol), the mixture was allowed to stir for 1.5 h at room temperature. The mixture was then diluted with EtOAc (100 ml) and washed with, respectively, sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (2 x 20 ml) and brine (20 ml). The organic layer was dried over MgSO<sub>4</sub> and concentrated *in vacuo*. Column chromatography (EtOAc/toluene/PE) afforded **7** (281 mg, 0.369 mmol, 74%) as a colourless oil. [α]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +3.2; IR: 737, 995, 1030, 1076, 1369, 1736, 2858, 2932; <sup>1</sup>H NMR (400 MHz): δ = 1.05 (s, 9H, *t*-Bu TBDPS), 3.17 (bs, 1H, OH), 3.53 - 3.68 (m, 5H, 2 x CHH glycerol, H-2, H-4, H-6), 3.72 - 3.90 (m, 4H, 2 x CHH glycerol, CH glycerol, H-5), 3.99 (dd, 1H, *J* = 4.9 Hz, 10.1 Hz, H-6), 4.04 (at, 1H, *J* = 9.3 Hz, H-3), 4.70 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.78 (d, 1H, *J* = 11.2 Hz, CHH Bn), 4.86 (d, 1H, *J* = 3.9 Hz, H-1), 4.88 (d, 1H, *J* = 11.7 Hz, CHH Bn), 4.93 (d, 1H, *J* = 11.2 Hz, CHH Bn), 5.50 (s, 1H, CH benzylidene), 7.25 - 7.46 (m, 21H, H<sub>arom</sub>), 7.63 - 7.66 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 19.1 (C<sub>q</sub> *t*-Bu, 26.8 (3 x CH<sub>3</sub> TBDPS), 62.7 (C-5), 62.9 (CH<sub>2</sub> glycerol), 63.9 (CH<sub>2</sub> glycerol), 68.8 (C-6), 74.5 (CH<sub>2</sub> Bn), 75.2 (CH<sub>2</sub> Bn), 78.9 (C-2, C-3), 81.8 (CH glycerol), 82.3 (C-4), 99.6 (C-1), 101.2 (CH benzylidene), 126.0 - 129.8 (CH<sub>arom</sub>), 133.0, 133.1 (2 x C<sub>q</sub> phenyl), 135.5 (CH<sub>arom</sub>), 137.3, 137.5, 138.5 (2 x C<sub>q</sub> Bn, C<sub>q</sub> benzylidene); HRMS: C<sub>46</sub>H<sub>52</sub>O<sub>8</sub>Si + Na<sup>+</sup> requires 783.3324, found 783.3325.



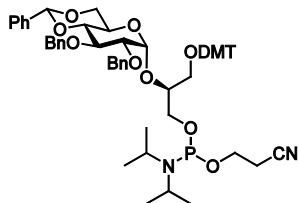
**1-O-(tert-Butyldiphenylsilyl)-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-sn-glycerol (8)**

To a solution of alcohol **7** (4.68 g, 6.14 mmol) in DCM (50 ml) were added, respectively, DiPEA (1.61 ml, 9.22 mmol) and DMT-Cl (2.50 g, 7.37 mmol). The mixture was allowed to stir overnight after which it was quenched by the addition of MeOH (5.0 ml). After stirring 15 min the mixture was washed with H<sub>2</sub>O (20 ml) and brine (20 ml) and the organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE/Et<sub>3</sub>N) yielded **8** (6.51 g, 6.12 mmol, 100%) as white foam. [α]<sub>D</sub><sup>20</sup> (MeOH): +17.2; IR: 1034, 1088, 1250, 1508, 2343, 2361; <sup>1</sup>H NMR (400 MHz): δ = 0.97 (s, 9H, *t*-Bu TBDPS), 3.30 (dd, 1H, *J* = 6.2 Hz, 9.8 Hz, CHH glycerol), 3.46 (dd, 1H, *J* = 4.4 Hz, 10.0 Hz), 3.52 - 3.64 (m, 3H, H-2, H-4, H-6), 3.72 - 3.80 (m, 8H, 2 x CHH glycerol, 2 x OMe), 3.90 - 3.97 (m, 1H, H-5), 4.00 - 4.07 (m, 3H, CH glycerol, H-3, H-6), 4.59 (d, 1H, *J* = 12.4 Hz, CHH Bn), 4.64 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.79 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.87 (d, 1H, *J* = 11.2 Hz, CHH Bn), 5.21 (d, 1H, *J* = 3.6 Hz, H-1), 5.50 (s, 1H, CH benzylidene), 6.74 - 6.76 (m, 4H, H<sub>arom</sub>), 7.12 - 7.46 (m, 30H, H<sub>arom</sub>), 7.58 - 7.62 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 19.1 (C<sub>q</sub> *t*-Bu, 26.8 (3 x CH<sub>3</sub> TBDPS), 55.1 (2 x OMe), 62.6 (C-5), 64.1 (2 x CH<sub>2</sub> glycerol), 68.9 (C-6), 72.5 (CH<sub>2</sub> Bn), 75.2 (CH<sub>2</sub> Bn), 77.5, 78.3 (CH glycerol, C-3), 78.9 (C-2), 82.2 (C-4), 86.5 (C<sub>q</sub> DMT), 97.2 (C-1), 101.3 (CH benzylidene), 113.1 (CH<sub>arom</sub>), 126.1 - 130.1 (CH<sub>arom</sub>), 133.2, 133.3 (2 x C<sub>q</sub> phenyl), 135.5 (CH<sub>arom</sub>), 136.0, 136.0, 137.6, 138.1, 138.8 (2 x C<sub>q</sub> Bn, C<sub>q</sub> benzylidene, 5 x C<sub>q</sub> DMT); HRMS: C<sub>67</sub>H<sub>70</sub>O<sub>10</sub>Si + H<sup>+</sup> requires 1063.4811, found 1063.4804.



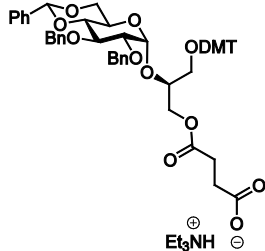
**2-O-(2,3-di-O-Benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-sn-glycerol (9)**

Compound **8** (6.33 g, 5.95 mmol) was dissolved in THF (60 ml) and after addition of TBAF (1M in THF, 10.7 ml) stirred overnight. After evaporation of the solvents under reduced pressure the resulting oil was purified by column chromatography (EtOAc/PE/Et<sub>3</sub>N), giving alcohol **9** (4.37 g, 5.30 mmol, 89%) as a white foam.  $[\alpha]_D^{20}$  (MeOH): +29.2; IR: 1032, 1076, 1250, 1508, 1609, 2343, 2361; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 2.40 (bs, 1H, OH), 3.28 (dd, 1H, *J* = 6.3 Hz, 9.8 Hz, CH<sub>2</sub> glycerol), 3.34 (dd, 1H, *J* = 5.4 Hz, 9.9 Hz, CH<sub>2</sub> glycerol), 3.54 (dd, 1H, *J* = 3.8 Hz, 9.4 Hz, H-2), 3.61 (at, 1H, *J* = 9.5 Hz, H-4), 3.66 - 3.77 (m, 9H, CH<sub>2</sub> glycerol, H-6, 2 x OMe), 3.87 (ddd, 1H, *J* = 3.5 Hz, 6.1 Hz, 11.8 Hz, CH glycerol), 3.98 (dd, 1H, *J* = 4.8 Hz, 10.0 Hz, H-5), 4.02 (at, 1H, *J* = 9.3 Hz, H-3), 4.25 (dd, 1H, *J* = 4.9 Hz, 10.2 Hz, H-6), 4.59 (d, 1H, *J* = 12.1 Hz, CH<sub>2</sub> Bn), 4.68 (d, 1H, *J* = 12.1 Hz, CH<sub>2</sub> Bn), 4.80 (d, 1H, *J* = 11.3 Hz, CH<sub>2</sub> Bn), 4.90 (d, 1H, *J* = 11.3 Hz, CH<sub>2</sub> Bn), 4.97 (d, 1H, *J* = 3.9 Hz, H-1), 5.54 (s, 1H, CH benzylidene), 6.80 (4, 4H, *J* = 8.9 Hz, H<sub>arom</sub>), 7.16 - 7.36 (m, 20H, H<sub>arom</sub>), 7.43 - 7.49 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 55.1 (2 x OMe), 62.9 (C-5), 63.3 (CH<sub>2</sub> glycerol), 63.9 (CH<sub>2</sub> glycerol), 68.9 (C-6), 73.2 (CH<sub>2</sub> Bn), 75.3 (CH<sub>2</sub> Bn), 78.4 (C-3), 78.8 (C-2), 78.8 (CH glycerol), 82.0 (C-4), 86.6 (C<sub>q</sub> DMT), 97.4 (C-1), 101.2 (CH benzylidene), 113.1 (CH<sub>arom</sub>), 126.0 - 130.0 (CH<sub>arom</sub>), 133.0 (2 x C<sub>q</sub> phenyl), 135.8, 137.3, 137.9, 138.7, 144.6, 158.5 (2 x C<sub>q</sub> Bn, C<sub>q</sub> benzylidene, 5 x C<sub>q</sub> DMT); HRMS: C<sub>51</sub>H<sub>52</sub>O<sub>10</sub> + Na<sup>+</sup> requires 847.3453, found 847.3455.



**1-O-([N,N-diisopropylamino]-2-cyanoethoxy-phosphite)-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-sn-glycerol (2a)**

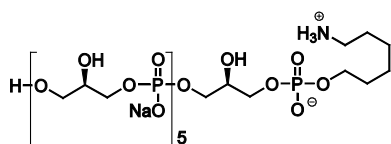
To a cooled (0 °C) solution of **9** (1.24 g, 1.50 mmol) and DiPEA (0.42 ml, 2.4 mmol) in freshly distilled DCM (30 ml) was added 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite (0.462 g, 1.95 mmol). After stirring overnight, the reaction was quenched by the addition of H<sub>2</sub>O (2.0 ml) and washed with H<sub>2</sub>O (10 ml) and brine (10 ml), respectively. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE/Et<sub>3</sub>N) gave phosphoramidite **2a** (1.50 g, 1.46 mmol, 98%) as white foam. <sup>31</sup>P NMR (161.7 MHz, CD<sub>3</sub>CN):  $\delta$  = 149.0, 149.3 (diastereoisomers); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN):  $\delta$  = 1.07 - 1.15 (m, 12H, 4 x CH<sub>3</sub> isopropylamino), 2.46 - 2.50 (m, 2H, CH<sub>2</sub> cyanoethyl), 3.11 - 3.29 (m, 2H, 2 x CH isopropylamino), 3.51 - 4.27 (m, 19H, CH<sub>2</sub> cyanoethyl, 2 x OMe, 2 x CH<sub>2</sub> glycerol, CH glycerol, H-2, H-3, H-4, H-5, H-6, H-6), 4.57 - 4.62 (m, 2H, CH<sub>2</sub> Bn), 4.79 (m, 2H, CH<sub>2</sub> Bn), 5.15 (m, 1H, H-1), 5.60 (s, 1H, CH benzylidene), 6.80 (d, 4H, *J* = 8.1 Hz, H<sub>arom</sub>), 7.11 - 7.47 (m, 24H, H<sub>arom</sub>); HRMS: C<sub>60</sub>H<sub>69</sub>N<sub>2</sub>O<sub>11</sub>P + H<sup>+</sup> requires 1025.4712, found 1025.4720.



**1-O-(Triethylammoniumsuccinate)-2-O-(2,3-di-O-benzyl-4,6-O-benzylidene-α-D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-sn-glycerol (2b)**

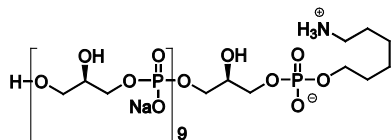
To a solution of **9** (3.21 g, 3.89 mmol) and Et<sub>3</sub>N (5.93 ml, 42.8 mmol) in DCM (40 ml) was added succinic anhydride (1.95 g, 19.45 mmol). A catalytic amount of DMAP was added and the reaction was stirred for 100 min. The mixture was diluted with DCM (50 ml) and washed with H<sub>2</sub>O (2 x 50 ml) after which the organic layer was concentrated under reduced pressure. The triethylammonium salt of succinyl ester **2b** (3.93 g, 3.83 mmol, 99%) was obtained as white foam.  $[\alpha]_D^{20}$  (MeOH): +31.6 °; IR: 829, 1034, 1250, 1508, 1738,

2343, 2361;  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{CN}$ ):  $\delta$  = 1.11 (t, 9H,  $J$  = 7.3 Hz, 3 x  $\text{CH}_3$   $\text{Et}_3\text{N}$ ), 2.36 - 2.39 (m, 2H,  $\text{CH}_2$  succinyl), 2.47 - 2.50 (m, 2H,  $\text{CH}_2$  succinyl), 2.82 (q, 6H,  $J$  = 7.3 Hz, 14.6 Hz, 3 x  $\text{CH}_2$   $\text{Et}_3\text{N}$ ), 3.21 (m, 2H,  $\text{CH}_2$  glycerol), 3.56 (dd, 1H,  $J$  = 3.7 Hz, 9.3 Hz, H-2), 3.64 (at, 1H,  $J$  = 9.5 Hz, H-4), 3.69 - 3.75 (m, 7H, 2 x OMe, H-6), 3.87 (at, 1H,  $J$  = 9.3 Hz, H-3), 3.94 - 4.00 (m, 2H, CH glycerol, H-5), 4.15 (dd, 1H,  $J$  = 6.4 Hz, 11.6 Hz,  $\text{CH}_2$  glycerol), 4.23 - 4.26 (m, 2H,  $\text{CH}_2$  glycerol, H-6), 4.58 (s, 2H,  $\text{CH}_2$  Bn), 4.79 (s, 2H,  $\text{CH}_2$  Bn), 5.10 (d, 1H,  $J$  = 3.7 Hz, H-1), 5.61 (s, 1H, CH benzylidene), 6.84 (d, 4H,  $J$  = 8.9 Hz,  $\text{H}_{\text{arom}}$ ), 7.14 - 7.50 (m, 24H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{CD}_3\text{CN}$ ):  $\delta$  = 9.5 (3 x  $\text{CH}_3$   $\text{Et}_3\text{N}$ ), 30.9 ( $\text{CH}_2$  succinyl), 31.7 ( $\text{CH}_2$  succinyl), 45.9 (3 x  $\text{CH}_2$   $\text{Et}_3\text{N}$ ), 55.9 (2 x OMe), 63.7 ( $\text{CH}_2$  glycerol), 63.7 (C-5), 65.0 ( $\text{CH}_2$  glycerol), 69.5 (C-6), 73.4 ( $\text{CH}_2$  Bn), 75.3 ( $\text{CH}_2$  Bn), 75.7 (CH glycerol), 79.0 (C-3), 80.3 (C-2), 82.6 (C-4), 87.3 ( $\text{C}_q$  DMT), 114.1 ( $\text{CH}_{\text{arom}}$ ), 127.2 - 131.0 ( $\text{CH}_{\text{arom}}$ ), 136.7, 136.8, 138.9, 139.4, 140.1, 146.0, 159.6 (2 x  $\text{C}_q$  Bn, 4 x  $\text{C}_q$  DMT,  $\text{C}_q$  benzylidene), 174.0, 176.8 (2 x CO succinate); HRMS (as free acid):  $\text{C}_{55}\text{H}_{56}\text{O}_{13} + \text{Na}^+$  requires 947.3613, found 947.3627.



### Hexaglycerolphosphate (16)

Synthesis on 10  $\mu\text{mol}$  scale (100 mg glycerol-CPG). Average coupling efficiency: 98.3% (5 couplings). Purification method A gave the semiprotected hexamer (**10**) as white amorphous solid (3.4 mg, 1.8  $\mu\text{mol}$ , 18%). Method B (synthesis on 15  $\mu\text{mol}$  scale) gave **10** as white amorphous solid (2.7 mg, 1.5  $\mu\text{mol}$ , 10%). LC-MS (gradient: 10 mM  $\text{NH}_4\text{OAc}$  in  $\text{H}_2\text{O}$ /acetonitrile 1/0  $\rightarrow$  1/9 in 13.5 min): r.t. 5.85 min,  $\text{C}_{74}\text{H}_{99}\text{NO}_{33}\text{P}_6 + \text{H}^+$  requires 1716.5 found 1716.6;  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.0 (4P), 1.1 (1P), 1.2 (1P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.12 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.26 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.42 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.92 (t, 2H,  $J$  = 6.9 Hz,  $\text{CH}_2$ -N hexylspacer), 3.50 - 3.61 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.68 - 3.93 (m, 29H,  $\text{CH}_2$ -O hexylspacer, 11 x  $\text{CH}_2$  glycerol, 5 x CH glycerol), 4.41 - 4.54 (m, 12H, 6 x  $\text{CH}_2$  Bn), 4.92 (s, 2H,  $\text{CH}_2$  benzylcarbamate), 7.16 - 7.32 (m, 35H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 25.6, 26.5, 29.7, 30.7 (4 x  $\text{CH}_2$  hexylspacer), 41.3 ( $\text{CH}_2$ -N hexylspacer), 61.4 ( $\text{CH}_2$  glycerol), 65.1, 65.4, 65.5 - 65.7, 67.2, 67.6 ( $\text{CH}_2$ -O hexylspacer, 11 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$  benzylcarbamate), 72.7, 73.0 (6 x  $\text{CH}_2$  Bn), 78.0 - 78.1 (5 x CH glycerol), 79.2 (CH glycerol), 128.6 - 129.7 ( $\text{CH}_{\text{arom}}$ ), 137.5, 138.4 - 138.5 (6 x  $\text{C}_q$  Bn,  $\text{C}_q$  benzylcarbamate), 159.2 ( $\text{C}_q$  benzylcarbamate); HRMS:  $[\text{C}_{74}\text{H}_{99}\text{NO}_{33}\text{P}_6 + 2\text{H}]^{2+}$  requires 858.7335, found 858.7340; Deprotection: The semiprotected hexamer (**10**, 4.7 mg, 2.5  $\mu\text{mol}$ ) was deprotected using the standard procedure yielding hexamer **16** (1.9 mg, 1.6  $\mu\text{mol}$ , 65%) as an amorphous off-white solid.  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.2 (1P), 1.2 (3P), 1.3 (2P);  $^1\text{H}$ -NMR (600 MHz):  $\delta$  = 1.36 - 1.38 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.58 - 1.64 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.94 (at, 2H,  $J$  = 7.5 Hz,  $\text{CH}_2$ -N hexylspacer), 3.54 (dd, 1H,  $J$  = 6.1 Hz, 11.8 Hz, CHH glycerol), 3.62 (dd, 1H,  $J$  = 4.2 Hz, 11.8 Hz, CHH glycerol), 3.78 - 3.91 (m, 25H,  $\text{CH}_2$ -O hexylspacer, 11 x  $\text{CH}_2$  glycerol, CH glycerol), 3.96 - 4.01 (m, 5H, 5 x CH glycerol);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 25.4, 26.1, 27.6, 30.3 (4 x  $\text{CH}_2$  hexylspacer), 40.4 ( $\text{CH}_2$ -N hexylspacer), 63.0 ( $\text{CH}_2$  glycerol), 66.9 - 67.3 ( $\text{CH}_2$ -O hexylspacer, 11 x  $\text{CH}_2$  glycerol), 70.4 - 70.6, 71.7 (6 x CH glycerol); HRMS:  $\text{C}_{24}\text{H}_{57}\text{NO}_{31}\text{P}_6 + \text{H}^+$  requires 1042.1413, found 1042.1425.

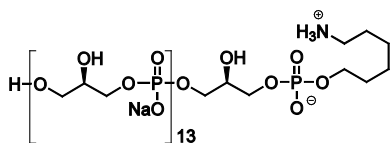


### 10-mer (17)

Synthesis on 10  $\mu\text{mol}$  scale (100 mg glycerol-CPG). Average coupling efficiency: 98.0% (9 couplings). Purification method A gave the semiprotected decamer (**11**) as white amorphous solid (8.5 mg, 2.9  $\mu\text{mol}$ , 29%). Method B (synthesis on 15  $\mu\text{mol}$  scale) gave the semiprotected decamer (**11**) as white amorphous solid (7.1 mg, 2.4  $\mu\text{mol}$ , 16%). LC-MS (gradient: 10 mM  $\text{NH}_4\text{OAc}$  in  $\text{H}_2\text{O}$ /acetonitrile 1/0  $\rightarrow$  1/9 in 13.5 min): r.t. 6.15 min,



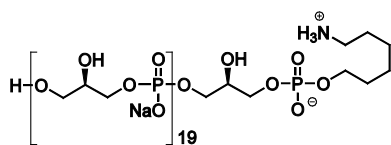
$[\text{C}_{114}\text{H}_{151}\text{NO}_{53}\text{P}_{10} + 2\text{H}]^{2+}$  requires 1346.8 found 1346.8;  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.0$  (8P), 1.1 (1P), 1.2 (1P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.03$  (m, 2H,  $\text{CH}_2$  hexylspacer), 1.08 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.20 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.38 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.86 (m, 2H,  $\text{CH}_2$ -N hexylspacer), 3.49 - 3.59 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.64 - 3.92 (m, 49H,  $\text{CH}_2$ -O hexylspacer, 19 x  $\text{CH}_2$  glycerol, 9 x CH glycerol), 4.33 - 4.47 (m, 20H, 10 x  $\text{CH}_2$  Bn), 4.82 (bs, 2H,  $\text{CH}_2$  benzylcarbamate), 7.00 - 7.22 (m, 55H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 25.7, 26.6, 29.8, 30.7$  (4 x  $\text{CH}_2$  hexylspacer), 41.2 ( $\text{CH}_2$ -N hexylspacer), 61.3 ( $\text{CH}_2$  glycerol), 65.1, 65.5 - 65.6, 67.1, 67.5 ( $\text{CH}_2$ -O hexylspacer, 19 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$  benzylcarbamate), 72.6, 72.8 - 72.9 (10 x  $\text{CH}_2$  Bn), 78.0 - 78.1 (9 x CH glycerol), 79.2 (CH glycerol), 128.6 - 129.6 ( $\text{CH}_{\text{arom}}$ ), 138.4 - 138.5 (10 x  $\text{C}_q$  Bn,  $\text{C}_q$  benzylcarbamate) 158.9 ( $\text{C}_q$  benzylcarbamate); HRMS:  $[\text{C}_{114}\text{H}_{151}\text{NO}_{53}\text{P}_{10} + 2\text{Na}]^{2+}$  requires 1369.3173, found 1369.3181; Deprotection: The partially protected decamer (**11**, 7.10 mg, 2.44  $\mu\text{mol}$ ) was deprotected using the standard procedure yielding decamer GTA **17** (3.6 mg, 1.9  $\mu\text{mol}$ , 78%) as an amorphous off-white solid.  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.2$  (1P), 1.3 (7P), 1.3 (2P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.36$  - 1.39 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.58 - 1.65 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.95 (at, 2H,  $J = 7.5$  Hz,  $\text{CH}_2$ -N hexylspacer), 3.55 (dd, 1H,  $J = 6.1$  Hz, 11.8 Hz, CHH glycerol), 3.62 (dd, 1H,  $J = 4.2$  Hz, 11.8 Hz, CHH glycerol), 3.72 (m, 1H, CHH glycerol), 3.80 - 4.02 (m, 49H,  $\text{CH}_2$ -O hexylspacer, 18 x  $\text{CH}_2$  glycerol, 1 x CHH glycerol, 10 x CH glycerol);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 25.4, 26.0, 27.5, 30.3$  (4 x  $\text{CH}_2$  hexylspacer), 40.3 ( $\text{CH}_2$ -N hexylspacer), 63.0 ( $\text{CH}_2$  glycerol), 66.9 - 67.3 ( $\text{CH}_2$ -O hexylspacer, 19 x  $\text{CH}_2$  glycerol), 70.4 - 70.5, 71.3, 71.6, 71.7 (10 x CH glycerol); HRMS:  $\text{C}_{36}\text{H}_{85}\text{NO}_{51}\text{P}_{10} + \text{H}^+$  requires 1658.1537, found 1658.1553.



#### 14-mer (**18**)

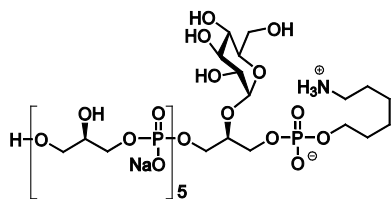
Synthesis on 10  $\mu\text{mol}$  scale (100 mg glycerol-CPG). Average coupling efficiency: 98.1% (13 couplings). Purification method A gave the semiprotected 14-mer (**12**) as white amorphous solid (3.0 mg, 0.75  $\mu\text{mol}$ , 8%). Method B gave the semiprotected 14-mer (**12**) as

white amorphous solid (8.2 mg, 2.1  $\mu\text{mol}$ , 21%). LC-MS (gradient: 10 mM  $\text{NH}_4\text{OAc}$  in  $\text{H}_2\text{O}$ /acetonitrile 1/0  $\rightarrow$  1/9 in 13.5 min): r.t. 5.84 min,  $[\text{C}_{154}\text{H}_{203}\text{NO}_{73}\text{P}_{14} + 3\text{H}]^{3+}$  requires 1224.0 found 1224.4;  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 0.7$  - 1.1 (14P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 0.99$  (m, 2H,  $\text{CH}_2$  hexylspacer), 1.05 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.15 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.35 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.83 (m, 2H,  $\text{CH}_2$ -N hexylspacer), 3.47 - 3.58 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.60 - 3.92 (m, 69H,  $\text{CH}_2$ -O hexylspacer, 27 x  $\text{CH}_2$  glycerol, 13 x CH glycerol), 4.29 - 4.44 (m, 28H, 14 x  $\text{CH}_2$  Bn), 4.72 (bs, 2H,  $\text{CH}_2$  benzylcarbamate), 6.91 - 7.15 (m, 75H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 24.7, 25.6, 28.9, 29.8$  (4 x  $\text{CH}_2$  hexylspacer), 40.3 ( $\text{CH}_2$ -N hexylspacer), 60.2 ( $\text{CH}_2$  glycerol), 64.1, 64.4 - 64.6, 66.1, 66.4 ( $\text{CH}_2$ -O hexylspacer, 27 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$  benzylcarbamate), 71.6, 71.8 (14 x  $\text{CH}_2$  Bn), 77.0 - 77.1 (13 x CH glycerol), 78.2 (CH glycerol), 127.6 - 128.6 ( $\text{CH}_{\text{arom}}$ ), 137.5 - 137.6 (14 x  $\text{C}_q$  Bn,  $\text{C}_q$  benzylcarbamate); HRMS:  $[\text{C}_{154}\text{H}_{203}\text{NO}_{73}\text{P}_{14} + 2\text{Na}]^{2+}$  requires 1857.4174 found 1857.4167; Deprotection: The semiprotected 14-mer (**12**, 5.5 mg, 1.4  $\mu\text{mol}$ ) was deprotected using the standard procedure yielding 14-mer glycerol TA **18** (3.0 mg, 1.2  $\mu\text{mol}$ , 84%) as an amorphous off-white solid.  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.1$  - 1.3 (14P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.37$  - 1.41 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.60 - 1.67 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.97 (at, 2H,  $J = 7.5$  Hz,  $\text{CH}_2$ -N hexylspacer), 3.57 (dd, 1H,  $J = 6.1$  Hz, 11.8 Hz, CHH glycerol), 3.64 (dd, 1H,  $J = 4.1$  Hz, 11.8 Hz, CHH glycerol), 3.71 - 3.76 (m, 1H, CHH glycerol), 3.78 - 4.04 (m, 69H,  $\text{CH}_2$ -O hexylspacer, 26 x  $\text{CH}_2$  glycerol, CHH glycerol, 14 x CH glycerol);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 25.4, 26.1, 27.6, 30.4$  (4 x  $\text{CH}_2$  hexylspacer), 40.4 ( $\text{CH}_2$ -N hexylspacer), 63.1 ( $\text{CH}_2$  glycerol), 65.8 ( $\text{CH}_2$  glycerol), 67.3 - 67.7 ( $\text{CH}_2$ -O hexylspacer, 26 x  $\text{CH}_2$  glycerol), 70.5 - 70.6, 71.3, 71.7 (14 x CH glycerol); HRMS:  $\text{C}_{48}\text{H}_{112}\text{NO}_{71}\text{P}_{14} + \text{H}^+$  requires 2273.1584, found 2273.1562.

**20-mer (19)**

Synthesis on 15  $\mu\text{mol}$  scale (150 mg glycerol-CPG). Average coupling efficiency: 98.5% (19 couplings). Purification method B gave the semiprotected 20-mer (**13**) as white amorphous solid (20.2 mg, 3.62  $\mu\text{mol}$ , 24%). LC-MS (gradient: 10 mM  $\text{NH}_4\text{OAc}$  in

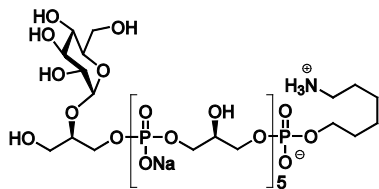
$\text{H}_2\text{O}/\text{acetonitrile}$  9/1  $\rightarrow$  1/9 in 13.5 min): r.t. 5.23 min,  $[\text{C}_{214}\text{H}_{281}\text{NO}_{103}\text{P}_{20} + 4\text{H}]^{4+}$  requires 1284.6 found 1284.8;  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 0.8 (1P), 1.0 (18P), 1.1 (1P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 0.96 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.03 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.13 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.33 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.82 (m, 2H,  $\text{CH}_2$ -N hexylspacer), 3.43 - 3.55 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.57 - 4.08 (m, 99H,  $\text{CH}_2$ -O hexylspacer, 39 x  $\text{CH}_2$  glycerol, 19 x CH glycerol), 4.17 - 4.44 (m, 40H, 20 x  $\text{CH}_2$  Bn), 4.69 (bs, 2H,  $\text{CH}_2$  benzylcarbamate), 6.85 - 7.13 (m, 105H,  $\text{H}_{\text{arom}}$ ), 7.68 (s, 1H, NH);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 25.7, 26.6, 30.0, 30.7 (4 x  $\text{CH}_2$  hexylspacer), 41.3 ( $\text{CH}_2$ -N hexylspacer), 61.1 ( $\text{CH}_2$  glycerol), 64.9 - 65.6, 67.1, 67.2, 67.6 ( $\text{CH}_2$ -O hexylspacer, 39 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$  benzylcarbamate), 72.5 - 72.8 (20 x  $\text{CH}_2$  Bn), 77.7 - 78.1 (19 x CH glycerol), 79.1 (CH glycerol), 128.6 - 129.6 ( $\text{CH}_{\text{arom}}$ ), 137.5, 138.4 - 138.7 (20 x  $\text{C}_q$  Bn,  $\text{C}_q$  benzylcarbamate), 158.4 ( $\text{C}_q$  benzylcarbamate); HRMS:  $[\text{C}_{214}\text{H}_{296}\text{N}_6\text{O}_{103}\text{P}_{20} + 3\text{H}]^{3+}$  (mass + 5 x  $\text{NH}_3$ ) requires 1740.7715, found 1740.7691; Deprotection: The partially protected 20-mer (**13**, 6.2 mg, 1.1  $\mu\text{mol}$ ) was deprotected using the standard procedure yielding 20-mer glycerol TA **19** (3.8 mg, 1.1  $\mu\text{mol}$ , 95%) as an amorphous off-white solid.  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.2 - 1.4 (20P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.36 - 1.40 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.59 - 1.71 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.95 (at, 2H,  $J$  = 7.5 Hz,  $\text{CH}_2$ -N hexylspacer), 3.56 (dd, 1H,  $J$  = 6.2 Hz, 11.8 Hz, CHH glycerol), 3.63 (dd, 1H,  $J$  = 4.3 Hz, 11.9 Hz, CHH glycerol), 3.72 (m, 1H, CHH glycerol), 3.81 - 4.04 (m, 99H,  $\text{CH}_2$ -O hexylspacer, 38 x  $\text{CH}_2$  glycerol, CHH glycerol, 20 x CH glycerol);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 25.4, 26.0, 27.6, 30.4 (4 x  $\text{CH}_2$  hexylspacer), 40.4 ( $\text{CH}_2$ -N hexylspacer), 63.0 ( $\text{CH}_2$  glycerol), 65.7 ( $\text{CH}_2$  glycerol), 66.9 - 67.5 ( $\text{CH}_2$ -O hexylspacer, 37 x  $\text{CH}_2$  glycerol), 67.7 ( $\text{CH}_2$  glycerol), 70.4 - 70.7, 71.3, 71.7 (20 x CH glycerol); HRMS:  $[\text{C}_{66}\text{H}_{155}\text{NO}_{101}\text{P}_{20} + 2\text{H}]^{2+}$  requires 1599.5961, found 1599.5971.

**glucosylated hexamer (20)**

Synthesis on 15  $\mu\text{mol}$  scale (150 mg glycerol-CPG). Average coupling efficiency: 98.2% (5 couplings). Purification method B gave the semiprotected hexamer (**14**) as white amorphous solid (10.5 mg, 4.86  $\mu\text{mol}$ , 32%). LC-MS (gradient: 10 mM  $\text{NH}_4\text{OAc}$  in  $\text{H}_2\text{O}/\text{acetonitrile}$  9/1  $\rightarrow$  1/1 in 25 min): r.t. 13.80 min,  $[\text{C}_{94}\text{H}_{119}\text{NO}_{38}\text{P}_6 + 2\text{H}]^{2+}$  requires 1029.3 found 1029.2;

$^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.0 (1P), 1.1 (3P), 1.1 (1P), 1.2 (1P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 0.85 - 1.17 (m, 6H, 3 x  $\text{CH}_2$  hexylspacer), 1.37 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.84 (m, 2H,  $\text{CH}_2$ -N hexylspacer), 3.34 - 4.11 (m, 38H,  $\text{CH}_2$ -O hexylspacer, 12 x  $\text{CH}_2$  glycerol, 6 x CH glycerol, H-2, H-3, H-4, H-5, 2 x H-6), 4.29 - 4.71 (m, 16H, 7 x  $\text{CH}_2$  Bn,  $\text{CH}_2$  benzylcarbamate), 5.22 (bs, 1H, H-1), 5.28 (bs, 1H, CH benzylidene), 6.86 - 7.43 (m, 45H,  $\text{H}_{\text{arom}}$ ); HRMS:  $[\text{C}_{94}\text{H}_{119}\text{NO}_{38}\text{P}_6 + \text{NH}_4 + \text{H}]^{2+}$  requires 1037.3123, found 1037.3120; Deprotection: The partially protected hexamer (**14**, 10.5 mg, 4.86  $\mu\text{mol}$ ) was deprotected using the standard procedure yielding hexamer monoglucosylglycerol TA **20** (4.4 mg, 3.3  $\mu\text{mol}$ , 68%) as an amorphous off-white solid.  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 0.9 (1P), 1.2 (3P), 1.3 (1P), 1.3 (1P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.36 - 1.40 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.58 - 1.65 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.94 (at, 2H,  $J$  = 7.5 Hz,  $\text{CH}_2$ -N hexylspacer), 3.34 (at, 1H,  $J$  = 9.6 Hz, H-4), 3.46 (dd, 1H,  $J$  = 3.7 Hz, 9.9 Hz, H-2), 3.55 (dd, 1H,  $J$  = 6.1 Hz, 11.8 Hz, CHH glycerol), 3.62 (dd, 1H,  $J$  = 4.2 Hz, 11.8 Hz, CHH glycerol), 3.69 - 3.72 (m, 3H, H-3, H-5, H-6), 3.79 - 4.00 (m, 30H,  $\text{CH}_2$ -O hexylspacer, 11 x  $\text{CH}_2$  glycerol, 5 x CH glycerol,

H-6), 4.05 (m, 1H, CH glycerol), 5.11 (d, 1H,  $J = 3.7$  Hz, H-1);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 25.4$ , 26.1, 27.6, 30.4 (4 x  $\text{CH}_2$  hexylspacer), 40.4 ( $\text{CH}_2\text{-N}$  hexylspacer), 61.5 (C-6), 63.0 ( $\text{CH}_2$  glycerol), 65.2 ( $\text{CH}_2$  glycerol), 66.1 ( $\text{CH}_2$  glycerol) 67.0 - 67.4 ( $\text{CH}_2\text{-O}$  hexylspacer, 9 x  $\text{CH}_2$  glycerol), 70.4 - 70.6 (4 x CH glycerol, C-4), 71.7 (CH glycerol), 72.5 (C-2), 72.8 (C-5), 73.9 (C-3), 76.3 (CH glycerol), 98.7 (C-1); HRMS:  $\text{C}_{30}\text{H}_{67}\text{NO}_{36}\text{P}_6 + \text{H}^+$  requires 1204.1941, found 1204.1957.



### glucosylated hexamer (**21**)

Synthesis on 15  $\mu\text{mol}$  scale (150 mg glucosylglycerol-CPG). Average coupling efficiency: 96.9% (5 couplings). Purification method B gave the semiprotected hexamer (**15**) as white amorphous solid (11.6 mg, 5.37  $\mu\text{mol}$ , 36%). LC-MS (gradient: 10 mM  $\text{NH}_4\text{OAc}$  in  $\text{H}_2\text{O}$ /acetonitrile 9/1  $\rightarrow$  1/1 in 25 min): r.t. 13.55 min,  $\text{C}_{94}\text{H}_{119}\text{NO}_{38}\text{P}_6 + \text{H}^+$  requires 2057.6 found 2058.0;  $^{31}\text{P}$ -

NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.0$  - 1.3 (6P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 0.90$  - 1.15 (m, 6H, 3 x  $\text{CH}_2$  hexylspacer), 1.33 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.80 (m, 2H,  $\text{CH}_2\text{-N}$  hexylspacer), 3.22 - 4.18 (m, 38H,  $\text{CH}_2\text{-O}$  hexylspacer, 12 x  $\text{CH}_2$  glycerol, 6 x CH glycerol, H-2, H-3, H-4, H-5, 2 x H-6), 4.30 - 4.69 (m, 16H, 7 x  $\text{CH}_2$  Bn,  $\text{CH}_2$  benzylcarbamate), 5.12 - 5.20 (m, 2H, H-1, CH benzylidene), 6.86 - 7.26 (m, 45H,  $\text{H}_{\text{arom}}$ ); HRMS:  $[\text{C}_{94}\text{H}_{119}\text{NO}_{38}\text{P}_6 + 2\text{H}]^{2+}$  requires 1028.7991, found 1028.7996; Deprotection: The partially protected hexamer (**15**, 2.2 mg, 0.99  $\mu\text{mol}$ ) was deprotected using the standard procedure yielding hexamer monoglucosyl TA **21** (1.1 mg, 0.85  $\mu\text{mol}$ , 86%) as an amorphous off-white solid.  $^{31}\text{P}$ -NMR (162 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.2$  (1P), 1.2 (3P), 1.3 (1P), 1.3 (1P);  $^1\text{H}$ -NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 1.36$  - 1.40 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.59 - 1.65 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.94 (at, 2H,  $J = 7.5$  Hz,  $\text{CH}_2\text{-N}$  hexylspacer), 3.36 (at, 1H,  $J = 9.7$  Hz, H-4), 3.48 (dd, 1H,  $J = 3.8$  Hz, 9.9 Hz, H-2), 3.68 - 3.73 (m, 4H, H-3, 2 x H-6,  $\text{CHH}$  glycerol), 3.77 - 4.02 (m, 32H,  $\text{CH}_2\text{-O}$  hexylspacer, 11 x  $\text{CH}_2$  glycerol,  $\text{CHH}$  glycerol, 6 x CH glycerol, H-5), 5.12 (d, 1H,  $J = 3.7$  Hz, H-1);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta = 25.4$ , 26.0, 27.6, 30.3 (4 x  $\text{CH}_2$  hexylspacer), 40.4 ( $\text{CH}_2\text{-N}$  hexylspacer), 61.5 ( $\text{CH}_2$  glycerol), 62.2 (C-6), 65.2 ( $\text{CH}_2$  glycerol), 66.9 - 67.2 ( $\text{CH}_2\text{-O}$  hexylspacer, 11 x  $\text{CH}_2$  glycerol), 70.4 - 70.6 (5 x CH glycerol, C-4), 72.4 (C-2), 72.9 (C-5), 73.8 (C-3), 77.8 (CH glycerol), 98.8 (C-1); HRMS:  $\text{C}_{30}\text{H}_{67}\text{NO}_{36}\text{P}_6 + \text{H}^+$  requires 1204.1941, found 1204.1956.

## References

1. Neuhaus, F.C.; Baddiley, J. *Microbiol. Mol. Biol. Rev.* **2003**, *67*, 686-723.
2. Weidenmaier, C.; Peschel, A. *Nat. Rev. Microbiol.* **2008**, *6*, 276-287.
3. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Fabretti, F.; Sange, T.; Holst, O.; Huebner, J. *Infect. Immun.* **2006**, *74*, 5703-5712.
4. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Sava, I.; Ye, L.; Bychowska, A.; Holst, O.; Huebner, J. *Plos One* **2011**, *6*, e17839.
5. Morath, S.; Geyer, A.; Hartung, T. *J. Exp. Med.* **2001**, *193*, 393-397.
6. Morath, S.; Stadelmaier, A.; Geyer, A.; Schmidt, R. R.; Hartung, T. *J. Exp. Med.* **2002**, *195*, 1635-1640.
7. Hashimoto, M.; Tawaratsumida, K.; Kariya, H.; Kiyohara, A.; Suda, Y.; Kirikae, F.; Kirikae, T.; Gotz, F. *J. Immunol.*, **2006**, *177*, 3162-3169.
8. von Aulock, S.; Hartung, T.; Hermann, C. *J. Immunol.* **2007**, *178*, 2610-2611.
9. Hashimoto, M.; Furuyashiki, M.; Suda, Y. *J. Immunol.* **2007**, *178*, 2610-2611.
10. Hogendorf, W.F.J.; Van den Bos, L.J.; Overkleeft, H.S.; Codée, J.D.C.; van der Marel, G.A. *Bioorg. Med. Chem.* **2010**, *18*, 3668-3678.
11. Caruthers, M.H. *Science* **1985**, *230*, 281-285.
12. Beaucage, S.L.; Iyer, R.P. *Tetrahedron* **1992**, *48*, 2223-2311.
13. Reese, C.B. *Org. Biomol. Chem.* **2005**, *3*, 3851-3868.
14. Pollack, J.H.; Neuhaus, F.C. *J. Bacteriol.* **1994**, *176*, 7252-7259.
15. Endl, J.; Seidl, H.P.; Fiedler, F.; Schleifer, K.H. *Arch. Microbiol.* **1983**, *135*, 215-
16. Hogendorf, W.F.J.; Meeuwenoord, N.; Overkleeft, H.S.; Filippov, D.V.; Laverde, D.; Kropec, A.; Huebner, J.; van der Marel, G.A.; Codée, J.D.C. *Chem. Commun.* **2011**, *47*, 8961-8963.
17. Schaller, H.; Weimann, G.; Lerch, B.; Khorana, H.G. *J. Am. Chem. Soc.* **1963**, *85*, 3821-3827.
18. Crich, D.; Cai, W. *J. Org. Chem.* **1999**, *64*, 4926-4930.
19. Crich, D.; Vinogradova, O. *J. Org. Chem.* **2006**, *71*, 8473-8480.
20. Crich, D. *Acc. Chem. Res.* **2010**, *43*, 1144-1153.
21. Codée, J.D.C.; van den Bos, L.J.; Litjens, R.E.J.N.; Overkleeft, H.S.; van Boom, J.H.; van der Marel, G.A. *Org. Lett.* **2003**, *5*, 1947-1950.
22. Oltvoort, J. J.; van Boeckel, C. A. A.; de Koning, J. H.; van Boom, J. H. *Synthesis* **1981**, 305-308.
23. Welz, R.; Müller, S. *Tetrahedron Lett.* **2002**, *43*, 795-797.
24. The use of the commercially available *N*-MMT-6-aminohexanol phosphoramidite led to the formation of *N*-cyanoethyl side products in the cleavage/deprotection step. Although this could be prevented by the use of 1,4-dithiothreitol (DTT) in the cleavage cocktail, we preferred the use of the stable benzyl carbamate **3**.
25. Pon, R.T. *Methods in Molecular Biology*; Agrawal, S., Ed.; Humana Press, Totowa, New Jersey, **1993**, Vol. 20, pp 465-496.



## Chapter 4

---

# Fluorous Linker Assisted TA Synthesis

### Introduction

**Chapter two** and **chapter three** describe the generation of teichoic acids (TAs) *via* solution phase and automated solid phase approaches, both of which have advantages and disadvantages. The solution phase protocol is relatively labor and time consuming due to the intermediate isolation and purification steps. However, the approach bears some important advantages: It can be executed on both small and large scale and it can be performed employing a stoichiometric amount or small excess of reagents.<sup>1</sup> The automated solid phase synthesis strategy is time and labor efficient by postponing product purification to the final stage of the synthesis, which makes it suitable for the generation of a small library of structures. The main disadvantages are that this protocol requires a relatively large excess of expensive building blocks (6-8 eq) and can only be executed on a relatively small scale (~15  $\mu$ mol).<sup>2</sup>

Alternative synthetic strategies have recently emerged that are based on the use of soluble supports. In an ideal situation, these strategies combine the “best of both worlds”: the soluble support allows the application of (a relatively small) excess of reagents to drive the reactions to completion; it also enables the rapid isolation and purification of intermediates and is readily adapted to different reaction scales. Several supports have been recommended over the years, including polyethylene glycol polymers (PEG)<sup>3</sup>, lipophilic tails<sup>4</sup> and ionic tags.<sup>5</sup> With the advent of fluorous solid phase extraction (F-SPE) methodologies, a technique known as light fluorous synthesis<sup>6-11</sup> has become popular for the construction of biopolymers, especially in the area of carbohydrate chemistry.<sup>10,12-16</sup> Applications in the assembly of oligopeptides<sup>10</sup> have also been reported, but the use of fluorous chemistry in oligonucleotide synthesis has been restricted to tagging techniques, in which a fluorous building block is employed at the end of a solid phase oligonucleotide synthesis to discriminate the target full length oligomers from unwanted, capped deletion sequences.<sup>17-19</sup>

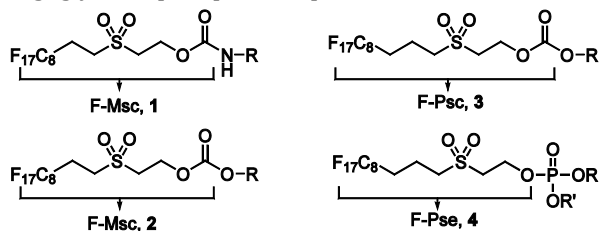
This chapter discusses the light fluorous approach to (aminoglucosylated) TAs. With use of perfluorooctylpropylsulfonyl ethyl (F-Pse) as the fluorous phosphate protection a dodecamer glycerol TA (**19**) is built up using phosphoramidite chemistry. After each elongation cycle, which comprises 3 steps (coupling, oxidation, detritylation), an F-SPE purification is performed. Using this technique a set of aminoglucosylated TA fragments is obtained (molecules **32a**, **32b** and **43**).<sup>20</sup> The

antigenicity of these fragments is determined using an opsonophagocytic inhibition assay (OPIA) which measures their binding to rabbit antibodies raised against enterococcal LTA.

## Results and Discussion

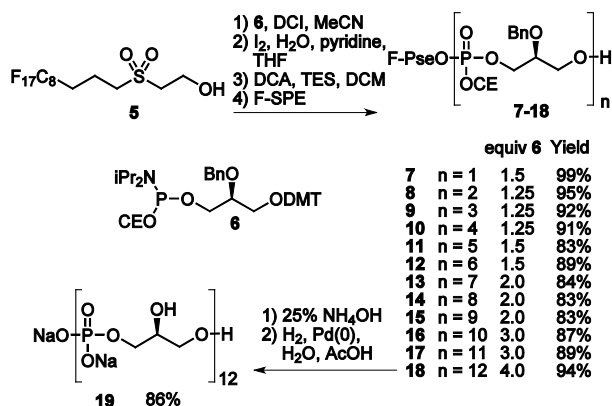
The first objective was the selection of a suitable light fluorine phosphate protecting group. To date only one such protecting group has been reported, which has been applied in the synthesis of a disaccharide.<sup>21</sup> In 2003 De Visser *et al.* and, more recently, Ali *et al.* reported on the use of fluorine sulfonyl ethyl based groups to protect both amino and hydroxyl functions, in the form of a carbamate and carbonate respectively (See Figure 1).<sup>22,23</sup> This group seemed suitable to protect phosphate functions since it can be removed at the end of the synthesis by base catalyzed  $\beta$ -elimination. The effective use of the 2-(methylsulfonyl) ethyl (MSc) group in solid phase oligonucleotide synthesis bodes well for this approach. Whereas the use of the fluorine version of the MSc group, the [1H,1H,2H,2H]-perfluorodecylsulfonyl-ethoxycarbonyl (F-Msc, **1**), functioned well as a nitrogen protecting group, it proved to be too base labile for use as a hydroxyl protecting group (as in **2**). Thus, for the fluorine version of the Msc carbonate, an extra methylene moiety between the fluorine part and the sulfonyl group was incorporated to provide extra insulation for the C<sub>8</sub>F<sub>17</sub> tail, giving the perfluorooctylpropylsulfonyl-ethoxycarbonyl (F-Psc, **3**) group.<sup>23</sup> Based on these considerations F-Pse group **4** was chosen as a fluorine linker and phosphate protecting group. With this linker, synthesized as reported previously, the first goal was to establish the scope and limitations of fluorine teichoic acid synthesis.

As depicted in Scheme 1, F-Pse alcohol **5** was elongated in a step-wise manner with glycerol phosphoramidite **6**.<sup>1</sup> Each elongation cycle consisted of four steps: 1) reaction of the alcohol with phosphoramidite **6** under the agency of dicyanoimidazole (DCI); 2) oxidation of the intermediate phosphite with I<sub>2</sub>; 3) removal of the DMT protecting group using dichloroacetic acid (DCA) in the presence of triethylsilane (TES); and 4) F-SPE purification. The work-up procedure includes an extraction with aqueous MeCN and hexane to remove most of the 4,4'-dimethoxytriphenylmethane and excess TES, since it was found that these could not be separated from the target compounds by F-SPE when present in relatively large amounts. Using this protocol, protected oligoglycerol phosphates up to the dodecamer level were rapidly generated. Although



the relative fluorine content in compounds **7-18** significantly decreases with increasing oligomer length (ranging from 37% fluorine in compound **7** to 8% fluorine in dodecamer **18**), this had little or no effect on

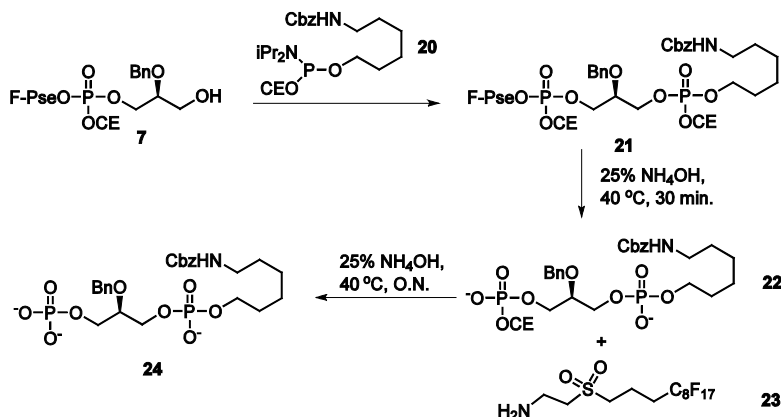
**Figure 1.** Fluorine versions of the MSc type protecting group.

**Scheme 1.** Light fluorous synthesis of dodecamer **19**.

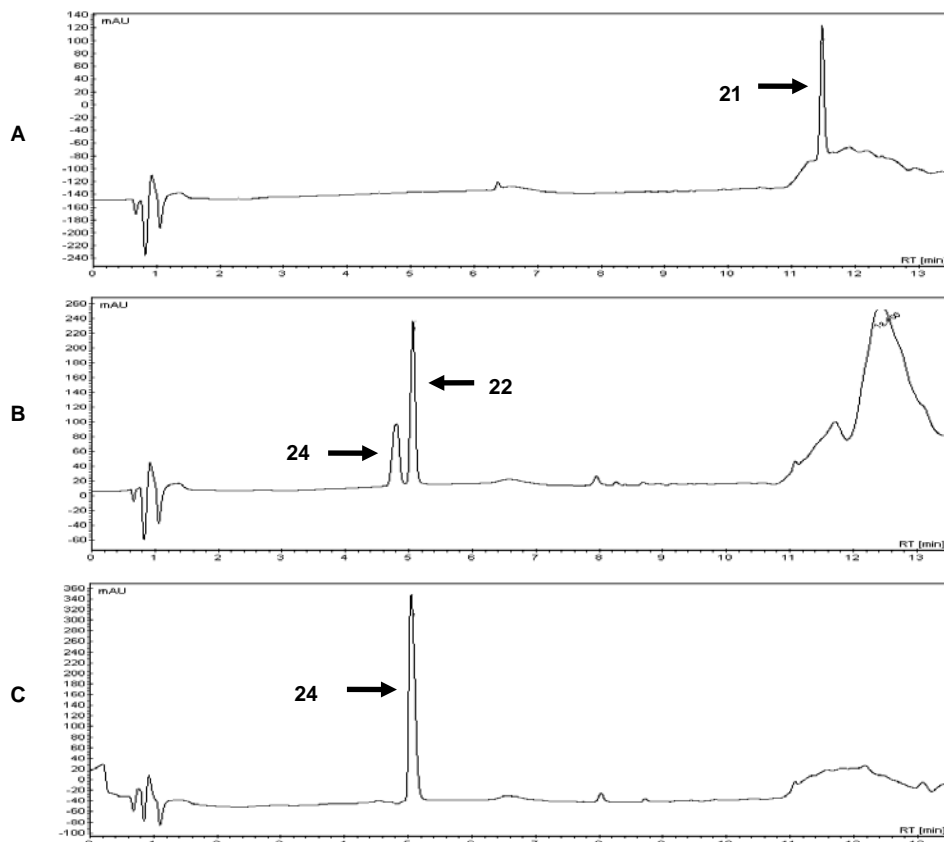
the purification efficiency and a single fluorous silica column (2 or 4g) was sufficient to purify all oligomers (0.1-0.25 mmol). However, a larger excess of **6** was required to push the coupling reactions to completion with increasing length of the oligomer. Up to four equivalents of the phosphoramidite were required in the final coupling

step to the dodecamer (see scheme 1). Because at this stage an automated solid phase approach becomes competitive, further elongation was abandoned.

Deprotection of the fully protected dodecamer (**18**) started with removal of the F-Pse and cyanoethyl groups using 25% aqueous ammonia solution. In a model deprotection experiment (Scheme 2) compound **21** was subjected to the above mentioned deprotection conditions at slightly elevated temperatures (40 °C) and it was observed that the F-Pse group at the terminal phosphotriester was selectively cleaved with respect to the cyanoethyl group (compound **22**, see Scheme 2 and Figure 2). Elimination of the remaining cyanoethyl group on the obtained phosphodiester required prolonged reaction times (typically overnight) to ensure complete unmasking of the target phosphomonoester (**24**). Applying this protocol to dodecamer **18** (scheme 1) led, after ensuing hydrogenolysis of the partially protected oligomer and gel filtration, to 30 mg of fully deprotected dodecamer **19** (86%).

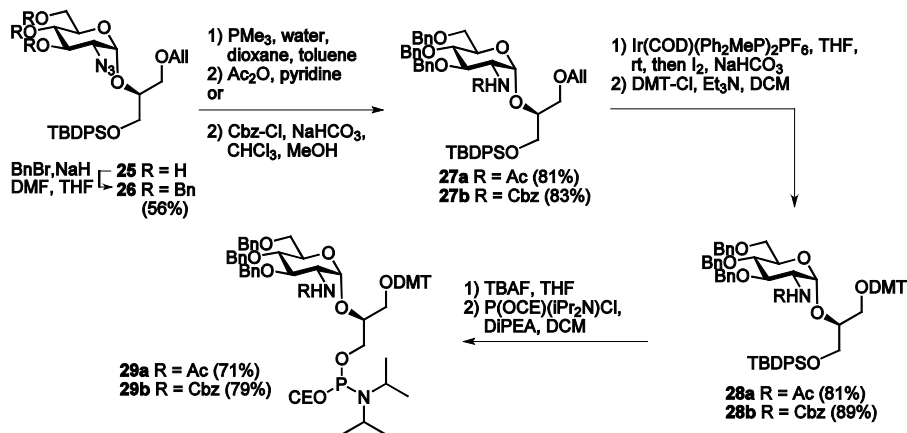
**Scheme 2.** Monitoring of the deprotection of model compound **21** with 25%  $\text{NH}_4\text{OH}$ .



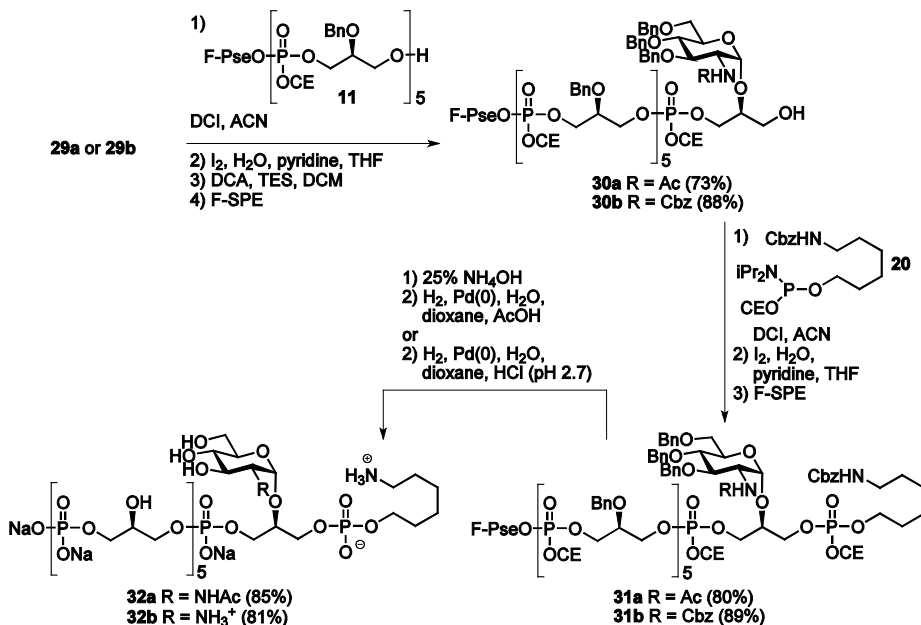


**Figure 2.** LC-MS (C-18, gradient (13.5 min) 10% → 90% MeCN, 15mM  $\text{NH}_4\text{OAc}$ ) analysis of the base catalyzed deprotection (25%  $\text{NH}_4\text{OH}$  at 40 °C) of compound **21**. A) before the reaction. B) after 30 minutes. C) after overnight reaction.

Having established that F-Pse alcohol **5** can be used for the efficient assembly of glycerol phosphate teichoic acids, the next goal was to investigate the incorporation of glycosyl substituents in the TA chains through the assembly of two teichoic acid hexamers. TA **32a** (Scheme 4) carries a GlcNAc residue, as present in TA chains of *Staphylococcus aureus*,<sup>24</sup> whereas a positively charged glucosamine is grafted on hexamer **32b**, a structural element found in several *Streptomyces* species.<sup>25,26</sup> The required glucosaminyl glycerol phosphoramidites were obtained as depicted in Scheme 3. Triol **25** was benzylated to give intermediate **26**<sup>27</sup> from which both building blocks **29a** and **29b** were assembled. Reduction of the azide functionality in **26** and subsequent acetylation gave *N*-acetyl glucosamine derivative **27a**, while protection with a benzyloxycarboxyl group led to **27b**. Both glucosaminyl glycerol building blocks were then transformed into the required phosphoramidites **29a** and **29b** following a well-established sequence of reactions, involving deallylation, dimethoxytritylation desilylation and phosphitylation (see Scheme 3).<sup>1,2,20</sup>

**Scheme 3.** Synthesis of glucosamineglycerol phosphoramidites **29a** and **29b**.

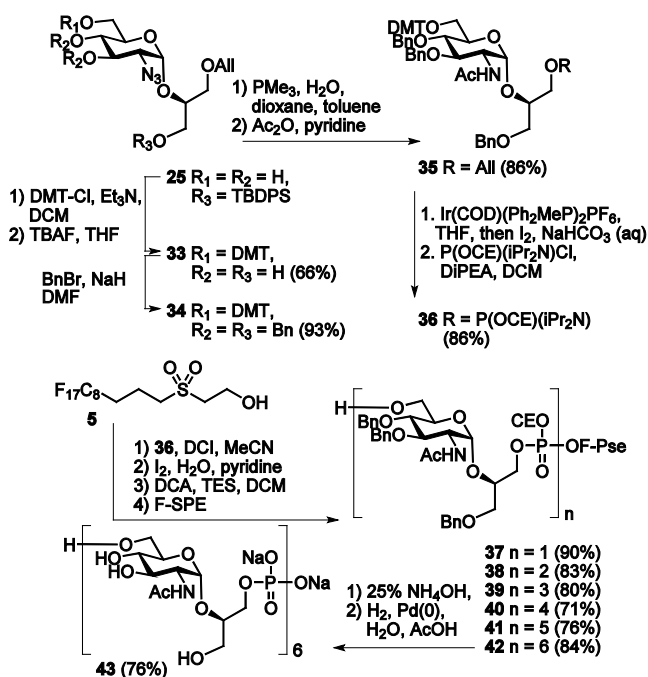
With building blocks **29a/b** the target hexamers **32a/b** were assembled starting from fluororous pentamer **11** (Scheme 4). Thus, condensation of **11** and **29a/b** and subsequent oxidation and removal of the DMT-group gave crude **30a/b**. Also in this case F-SPE purification proceeded uneventfully and hexamers **30a/b** were obtained in 73% and 88% respectively. The hexamers were then equipped with a hexylamino spacer using phosphoramidite **20** to give fully protected TA structures **31a/b** in pure form after F-SPE. Global deprotection using the deprotection protocol described above led to both target hexamers **32a/b**, the successful assembly of which indicates that the

**Scheme 4.** Light fluororous synthesis of glucosamine containing GTA hexamer **32a** and **32b**.

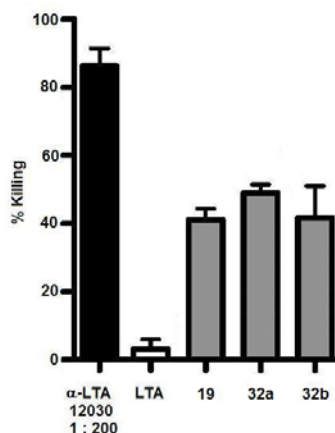
fluorous synthesis strategy can also be applied to substituted oligoglycerolphosphates.

Finally, the assembly of more complex structures was explored, as exemplified by the synthesis of teichoic acid fragment **43**, which is characterized by the [ $\rightarrow$ 6]-glucosamine-( $\alpha$ -1 $\rightarrow$ 2)-sn-glycerol-1-phosphate-] repeating unit and is found in *Spirilliplanes Yamanashiensis* (scheme 5).<sup>28</sup> The synthesis of the required GlcNHAc-glycerol phosphoramidite building block **36** commenced with dimethoxytritylation of the primary alcohol in **25**. Subsequent desilylation and benzylation of the resulting triol gave the fully protected GlcNHAc-glycerol **34**, which was transformed into required phosphoramidite **36** through azide reduction and acetylation followed by a deallylation-phosphitylation reaction sequence. For the assembly of hexamer **43**, F-Pse linker **5** was elongated in a step-wise manner with **36** using the chemistry described above. As can be seen in Scheme 5 all elongation steps proceeded efficiently. Although **36** is a more lipophilic building block than the above described glycerol phosphates, this did not pose any problems in the purification of the oligomers. Notably, the single C<sub>8</sub>F<sub>17</sub>-tail sufficed for the easy purification of fully protected hexamer **42**, having a molecular mass of 4.7 kDa (relative fluorine content: 7%). Finally, deprotection of **42** was accomplished by  $\beta$ -elimination of the F-Pse linker and cyanoethyl groups and global debenzoylation to give target compound **43** in 76% yield.

**Scheme 5.** Synthesis of complex hexamer **43**.



TA fragments **19**, **32a** and **32b** were evaluated in an OPIA, where the binding of the molecules to antibodies raised in rabbits against enterococcal LTA was measured. The three TAs all partially inhibited the killing of *E. faecalis* by the opsonic antibodies when added in a concentration of 100  $\mu\text{g}/\text{ml}$  (see Figure 3). However, when compared to the glucosylated hexamers described in **chapter three** the inhibition of killing by these molecules was



**Figure 3.** Results OPIA of compounds **19**, **32a/b** at 100  $\mu$ g/ml. The left bar represents killing by the serum without addition of inhibitor. The second bar from the left (LTA) represents the positive control where native LTA is added as the inhibitor.

considerably less. This indicates that the presence of either the (*N*-acetyl)aminoglucosyl or the terminal phosphate moiety (or both) might have a negative effect on the potency of the antigen.

## Conclusion

In conclusion, an efficient fluorous synthesis strategy for the assembly of teichoic acid fragments, was developed, based on the application of perfluorooctylpropylsulfonylethanol as a new fluorous phosphate protecting group. The strategy is especially useful for the assembly of multi-milligram quantities of medium sized TA fragments, featuring 6-12 repeating units. As displayed by the assembly of teichoic acid fragment **43**, complex glycerol phosphate building blocks can also be used, indicating that this strategy might be a valuable asset for the construction of various classes of phosphate ester containing biomolecules.

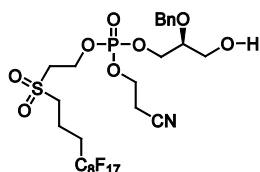
## Experimental section

**General Procedures and Material:** All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma-Aldrich, Genscript, Fluorous Technologies) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20%  $\text{H}_2\text{SO}_4$  in ethanol or with a solution of  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4 \text{H}_2\text{O}$  25 g/l and  $(\text{NH}_4)_4\text{Ce}(\text{SO}_4)_4 \cdot 2 \text{H}_2\text{O}$  10 g/l, in 10% aqueous  $\text{H}_2\text{SO}_4$  followed by charring at  $\pm 140^\circ\text{C}$ . Some unsaturated compounds were visualized by spraying with a solution of  $\text{KMnO}_4$  (2%) and  $\text{K}_2\text{CO}_3$  (1%) in water. Optical rotation measurements ( $[\alpha]_{\text{D}}^{20}$ ) were performed on a Propol automated polarimeter (Sodium D-line,  $\lambda = 589 \text{ nm}$ ) with a concentration of 10 mg/ml ( $c = 1$ ), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300.  $^{31}\text{P}$ ,  $^1\text{H}$ , and  $^{13}\text{C}$  NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in  $\text{CDCl}_3$  with chemical shift ( $\delta$ ) relative to tetramethylsilane, unless stated otherwise. When  $\text{D}_2\text{O}$  was used,  $^1\text{H}$ -NMR spectra were recorded with chemical shift relative ( $\delta$ ) to HDO (4.755 ppm),  $^{31}\text{P}$  spectra were measured with chemical shift relative to 85%  $\text{H}_3\text{PO}_4$  (external standard) and  $^{13}\text{C}$ -NMR spectra were recorded with chemical shift relative to TMS (external standard). High resolution mass spectra (HRMS) were recorded by direct injection (2  $\mu\text{l}$  of a 2  $\mu\text{M}$  solution in water/acetonitrile; 50/50; v/v and either 0.1% formic acid or 10mM ammonium formate for the oligomers) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature  $275^\circ\text{C}$ ) with resolution  $R = 60000$  at  $m/z$  400 (mass range  $m/z = 150\text{--}2000$ ) and dioctylphthalate ( $m/z = 391.28428$ ) as a

lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

**General procedure for phosphoramidite coupling, oxidation, detritylation and F-SPE on a typical scale (0.1-0.25 mmol):** Starting alcohol was dissolved in MeCN (0.1M). DCI (0.25M solution in MeCN, 2 eq with respect to phosphoramidite) was added, together with freshly activated MS3Å and the mixture was stirred under argon for 15 minutes. Phosphoramidite (0.1-0.2 M solution in MeCN, 1.3 - 4.0 eq with respect to starting material) was added and the reaction was stirred until TLC analysis revealed full conversion of the starting material into a higher running spot (~1 hr). Added were H<sub>2</sub>O (~1 ml) and I<sub>2</sub> (0.2 M in THF/pyr 4/1, 1.5 eq with respect to phosphoramidite) and the mixture was stirred for an additional 5 min. The mixture was diluted with EtOAc (~50 ml) and washed with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (~20 ml), 0.5 M KHSO<sub>4</sub> (~20 ml) and a 1/1 mixture of sat. aq. NaHCO<sub>3</sub> and brine (~20 ml), respectively. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> (s) and concentrated under reduced pressure. The residue was coevaporated once with toluene (10 ml) before it was redissolved in DCM (50 mM). Triethylsilane and dichloroacetic acid (20 eq with respect to starting material) were added and the mixture was stirred until the bright orange color fully disappeared (~30 min). DCM (~40 ml) was added and the organic layer was washed with a 1/1 mixture of sat. aq. NaHCO<sub>3</sub> and brine (~20 ml, check if pH >7), before it was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The residue was taken up in 4/1 MeCN/H<sub>2</sub>O (10 ml) and washed with hexane (50 ml). The hexane layer was extracted twice with 4/1 MeCN/H<sub>2</sub>O (2 x 10 ml) and the combined MeCN/H<sub>2</sub>O layers were concentrated under reduced pressure in a 100 ml pear shaped flask. The residue was taken up in 0.5 ml MeCN and applied to a small column containing fluoroflash™ fluorosilica (2 or 4g) which was preeluted with 1/1 MeCN/H<sub>2</sub>O. The column was eluted with 1/1 MeCN/H<sub>2</sub>O until all the non-fluorous byproducts (DMT-H, phosphates, DCI) were removed. Subsequently the fluorosilica product was eluted from the column with CH<sub>3</sub>CN and acetone.

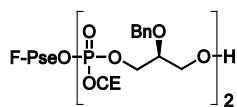
**Global deprotection and purification of oligomers:** The fully protected oligomer was treated with a 9/1 mixture of 28% NH<sub>4</sub>OH (aq)/1,4-dioxane at a concentration of 5 mg/ml at 40 - 45 °C overnight in a sealed flask or tube. After cooling down to RT the mixture was washed with Et<sub>2</sub>O (equal volume) and the ether layer was extracted twice with H<sub>2</sub>O (~5.0 ml). The aqueous layer was concentrated under reduced pressure after which NMR and HRMS analysis confirmed full conversion to the semiprotected intermediate. The intermediate was then treated with Pd (0)/H<sub>2</sub> (using ~5 mg palladium black per ml reaction medium) in a slightly acidic (pH ~2.7) mixture of dioxane/water (1/4, containing ~1% AcOH, or, in the case of hexamer **27b**, containing aqueous HCl, pH 2.7) at a concentration of ~5 mg of starting material per ml. After stirring for three days the mixture was filtered and concentrated *in vacuo*. The residue was purified by size exclusion chromatography (Sephadex HW40, eluent: 0.15 M NH<sub>4</sub>OAc). After repeated lyophilisation, the purified product was eluted through a small column containing Dowex Na<sup>+</sup> cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H<sub>2</sub>O, flushed with H<sub>2</sub>O and MeOH before use). Lyophilization gave the fully deprotected oligomer of which the integrity and purity was confirmed by HRMS and NMR (<sup>1</sup>H, <sup>13</sup>C, <sup>31</sup>P) analysis.



**1-[(Perfluorooctylpropylsulfonyl)ethyl]-2-(2-cyanoethyl)-phosphate]-2-O-benzyl-*sn*-glycerol (**7**)**

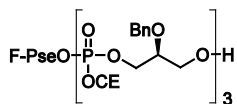
Perfluorooctylpropylsulfonyl ethanol **5** (145 mg, 254 μmol) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 2.18 ml, 381 μmol, 1.5 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Monomer **7** (218 mg, 251 μmol, 99 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7

MHz):  $\delta = -1.5, -1.5$  (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta = 2.12 - 2.36$  (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 2.52 (bs, 1H,  $\text{CH}_2\text{OH}$ ), 2.66 - 2.72 (m, 2H,  $\text{CH}_2$  cyanoethyl), 3.08 - 3.15 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 3.29 - 3.35 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 3.68 - 3.77 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 4.19 - 4.27 (m, 3H, CHH glycerol,  $\text{CH}_2$  cyanoethyl), 4.31 - 4.38 (m, 1H, CHH glycerol), 4.47 - 4.52 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 4.62 - 4.68 (m, 2H,  $\text{CH}_2$  Bn), 7.30 - 7.37 (m, 5H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 13.4$  ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 19.5 - 19.6 ( $\text{CH}_2$  cyanoethyl), 29.3 (t,  $J = 22$  Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 53.1 - 53.3 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 60.5 ( $\text{CH}_2$  glycerol), 61.3 - 61.4 ( $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 62.3 - 62.4 ( $\text{CH}_2$  cyanoethyl), 66.8 - 66.9 ( $\text{CH}_2$  glycerol), 72.0 ( $\text{CH}_2$  Bn), 77.4 (CH glycerol), 116.6 ( $\text{C}_q$  cyanoethyl), 127.9 - 128.5 ( $\text{CH}_{\text{arom}}$ ), 137.5 ( $\text{C}_q$  Bn); HRMS:  $\text{C}_{26}\text{H}_{27}\text{F}_{17}\text{NO}_8\text{P}_2\text{S} + \text{H}^+$  requires 868.0996, found 868.0996.



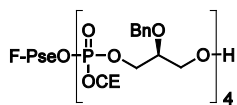
### 2-O-Benzyl-glycerol phosphate dimer (8)

Alcohol **7** (217 mg, 250  $\mu\text{mol}$ ) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 1.86 ml, 325  $\mu\text{mol}$ , 1.3 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. dimer **8** (277 mg, 238  $\mu\text{mol}$ , 95 %) was obtained as a colorless oil.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta = -1.9, -1.9, -1.8, -1.8$  (1P), -0.8, -0.8, -0.8, -0.8 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta = 2.11 - 2.35$  (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 2.56 - 2.70 (m, 5H, 2 x  $\text{CH}_2$  cyanoethyl,  $\text{CH}_2\text{OH}$ ), 3.07 - 3.14 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 3.26 - 3.34 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 3.65 - 3.78 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.82 - 3.86 (m, 1H, CH glycerol), 4.15 - 4.36 (m, 10H, 3 x  $\text{CH}_2$  glycerol, 2 x  $\text{CH}_2$  cyanoethyl), 4.45 - 4.51 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 4.59 - 4.67 (m, 4H, 2 x  $\text{CH}_2$  Bn), 7.28 - 7.37 (m, 10H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 13.3$  ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 19.4 - 19.5 (2 x  $\text{CH}_2$  cyanoethyl), 29.3 (t,  $J = 22$  Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 53.0 - 53.2 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 60.4 - 60.5 ( $\text{CH}_2$  glycerol), 61.3 - 61.4 ( $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 62.1 ( $\text{CH}_2$  cyanoethyl), 62.4 ( $\text{CH}_2$  cyanoethyl), 65.3 ( $\text{CH}_2$  glycerol), 66.0 ( $\text{CH}_2$  glycerol), 66.5 - 66.6 ( $\text{CH}_2$  glycerol), 66.8 - 66.9 ( $\text{CH}_2$  glycerol), 72.0 ( $\text{CH}_2$  Bn), 72.2 ( $\text{CH}_2$  Bn), 75.1 - 75.2 (CH glycerol), 77.4 - 77.5 (CH glycerol), 116.6 (2 x  $\text{C}_q$  cyanoethyl), 127.8 - 128.5 ( $\text{CH}_{\text{arom}}$ ), 137.0 ( $\text{C}_q$  Bn), 137.6 ( $\text{C}_q$  Bn); HRMS:  $\text{C}_{39}\text{H}_{43}\text{F}_{17}\text{N}_2\text{O}_{13}\text{P}_2\text{S} + \text{H}^+$  requires 1165.1762, found 1165.1756.



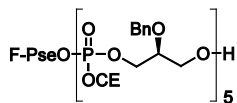
### 2-O-Benzyl-glycerol phosphate trimer (9)

Glycerol phosphate dimer **8** (275 mg, 236  $\mu\text{mol}$ ) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 2.02 ml, 354  $\mu\text{mol}$ , 1.5 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. trimer **9** (318 mg, 217  $\mu\text{mol}$ , 92 %) was obtained as a colorless oil.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta = -1.9, -1.9, -1.8, (1\text{P}), -1.3, -1.3, -1.2, -1.1$  (1P), -0.8, -0.8, -0.8, -0.8 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta = 2.10 - 2.36$  (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 2.56 - 2.73 (m, 7H, 3 x  $\text{CH}_2$  cyanoethyl,  $\text{CH}_2\text{OH}$ ), 3.06 - 3.13 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 3.26 - 3.34 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 3.64 - 3.73 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.81 - 3.86 (m, 2H, 2 x CH glycerol), 4.11 - 4.35 (m, 16H, 5 x  $\text{CH}_2$  glycerol, 3 x  $\text{CH}_2$  cyanoethyl), 4.44 - 4.50 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 4.58 - 4.67 (m, 6H, 3 x  $\text{CH}_2$  Bn), 7.27 - 7.37 (m, 15H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 13.3$  ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 19.3 - 19.4 (3 x  $\text{CH}_2$  cyanoethyl), 29.2 (t,  $J = 22$  Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 53.0 - 53.2 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 60.3 - 60.4 ( $\text{CH}_2$  glycerol), 61.3 ( $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 62.0 - 62.4 (3 x  $\text{CH}_2$  cyanoethyl), 65.4 - 66.6 (5 x  $\text{CH}_2$  glycerol), 71.9 ( $\text{CH}_2$  Bn), 72.1 (2 x  $\text{CH}_2$  Bn), 75.2 - 75.3 (2 x CH glycerol), 77.4 - 77.5 (CH glycerol), 116.7 (3 x  $\text{C}_q$  cyanoethyl), 127.5 - 128.5 ( $\text{CH}_{\text{arom}}$ ), 137.1 (2 x  $\text{C}_q$  Bn), 137.7 ( $\text{C}_q$  Bn); HRMS:  $\text{C}_{52}\text{H}_{59}\text{F}_{17}\text{N}_3\text{O}_{18}\text{P}_3\text{S} + \text{Na}^+$  requires 1484.2348, found 1484.2363.



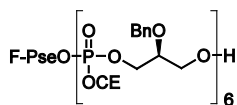
### 2-O-Benzyl-glycerol phosphate tetramer (10)

Glycerol phosphate trimer **9** (311 mg, 213  $\mu\text{mol}$ ) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 1.83 ml, 320  $\mu\text{mol}$ , 1.5 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. tetramer **10** (339 mg, 192  $\mu\text{mol}$ , 91 %) was obtained as a colorless oil.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta$  = -1.9, -1.9 (1P), -1.4, -1.3 (1P), -1.2, -1.2, -1.1 (1P), -0.9, -0.9, -0.8, -0.8 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 2.10 - 2.36 (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 2.54 - 2.76 (m, 9H, 4 x  $\text{CH}_2$  cyanoethyl,  $\text{CH}_2\text{OH}$ ), 3.06 - 3.13 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 3.25 - 3.34 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 3.64 - 3.72 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.79 - 3.85 (m, 3H, 3 x CH glycerol), 4.10 - 4.35 (m, 22H, 7 x  $\text{CH}_2$  glycerol, 4 x  $\text{CH}_2$  cyanoethyl), 4.44 - 4.50 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 4.58 - 4.67 (m, 8H, 4 x  $\text{CH}_2$  Bn), 7.27 - 7.36 (m, 20H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 13.3 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 19.3 - 19.4 (4 x  $\text{CH}_2$  cyanoethyl), 29.2 (t,  $J$  = 22 Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 52.9 - 53.1 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 60.3 - 60.4 ( $\text{CH}_2$  glycerol), 61.3 ( $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 62.0 - 62.4 (4 x  $\text{CH}_2$  cyanoethyl), 65.4 - 66.6 (7 x  $\text{CH}_2$  glycerol), 71.9 ( $\text{CH}_2$  Bn), 72.1 (3 x  $\text{CH}_2$  Bn), 75.1 - 75.4 (3 x CH glycerol), 77.4 - 77.5 (CH glycerol), 116.6 - 116.7 (4 x  $\text{C}_q$  cyanoethyl), 127.7 - 128.5 ( $\text{CH}_{\text{arom}}$ ), 137.1 - 137.2 (3 x  $\text{C}_q$  Bn), 137.7 ( $\text{C}_q$  Bn); HRMS:  $\text{C}_{65}\text{H}_{75}\text{F}_{17}\text{N}_4\text{O}_{23}\text{P}_4\text{S} + \text{Na}^+$  requires 1781.3114, found 1781.3106.



### 2-O-Benzyl-glycerol phosphate pentamer (11)

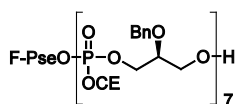
Glycerol phosphate tetramer **10** (538 mg, 306  $\mu\text{mol}$ ) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 2.62 ml, 459  $\mu\text{mol}$ , 1.5 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Pentamer **11** (524 mg, 255  $\mu\text{mol}$ , 83 %) was obtained as a colorless oil.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta$  = -1.9, -1.9 (1P), -1.4 - -1.1 (3P), -0.9, -0.9, -0.9 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 2.11 - 2.34 (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 2.53 - 2.70 (m, 11H, 5 x  $\text{CH}_2$  cyanoethyl,  $\text{CH}_2\text{OH}$ ), 3.06 - 3.13 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 3.25 - 3.34 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 3.63 - 3.72 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.79 - 3.85 (m, 4H, 4 x CH glycerol), 4.09 - 4.34 (m, 28H, 9 x  $\text{CH}_2$  glycerol, 5 x  $\text{CH}_2$  cyanoethyl), 4.43 - 4.50 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 4.58 - 4.66 (m, 10H, 5 x  $\text{CH}_2$  Bn), 7.26 - 7.36 (m, 25H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 13.3 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 19.2 - 19.4 (5 x  $\text{CH}_2$  cyanoethyl), 29.2 (t,  $J$  = 21 Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 52.9 - 53.1 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 60.3 - 60.4 ( $\text{CH}_2$  glycerol), 61.3 ( $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 62.0 - 62.4 (5 x  $\text{CH}_2$  cyanoethyl), 65.4 - 66.6 (9 x  $\text{CH}_2$  glycerol), 71.8 ( $\text{CH}_2$  Bn), 72.1 (4 x  $\text{CH}_2$  Bn), 75.1 - 75.3 (4 x CH glycerol), 77.4 - 77.5 (CH glycerol), 116.6 - 116.7 (5 x  $\text{C}_q$  cyanoethyl), 127.7 - 128.5 ( $\text{CH}_{\text{arom}}$ ), 137.1 - 137.2 (4 x  $\text{C}_q$  Bn), 137.7 ( $\text{C}_q$  Bn); HRMS:  $[\text{C}_{78}\text{H}_{91}\text{F}_{17}\text{N}_5\text{O}_{28}\text{P}_5\text{S} + 2\text{NH}_4]^{2+}$  requires 1045.7332, found 1045.7344.



### 2-O-Benzyl-glycerol phosphate hexamer (12)

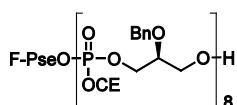
Glycerol phosphate pentamer **11** (237 mg, 115  $\mu\text{mol}$ ) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 0.99 ml, 173  $\mu\text{mol}$ , 1.5 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Hexamer **12** (240 mg, 102  $\mu\text{mol}$ , 89 %) was obtained as a colorless oil.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta$  = -1.9, -1.9 (1P), -1.4 - -1.1 (4P), -0.9, -0.9, -0.9 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 2.09 - 2.36 (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 2.46 - 2.69 (m, 13H, 6 x  $\text{CH}_2$  cyanoethyl,  $\text{CH}_2\text{OH}$ ), 3.06 - 3.13 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 3.25 - 3.34 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 3.63 - 3.72 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.79 - 3.86 (m, 5H, 5 x CH glycerol), 4.08 - 4.33 (m, 34H, 11 x  $\text{CH}_2$  glycerol, 6 x  $\text{CH}_2$  cyanoethyl), 4.43 - 4.50 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 4.58 - 4.66 (m, 12H, 6 x  $\text{CH}_2$  Bn), 7.26 - 7.36 (m, 30H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 13.3 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 19.2 - 19.4 (6 x  $\text{CH}_2$  cyanoethyl), 29.3 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 53.0 - 53.1 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 60.4

- 60.5 (CH<sub>2</sub> glycerol), 61.3 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 62.0 - 62.4 (6 x CH<sub>2</sub> cyanoethyl), 65.4 - 66.6 (11 x CH<sub>2</sub> glycerol), 71.9 (CH<sub>2</sub> Bn), 72.1 (5 x CH<sub>2</sub> Bn), 75.1 - 75.4 (5 x CH glycerol), 77.5 - 77.6 (CH glycerol), 116.7 - 116.8 (6 x C<sub>q</sub> cyanoethyl), 127.8 - 128.5 (CH<sub>arom</sub>), 137.1 - 137.3 (5 x C<sub>q</sub> Bn), 137.7 (C<sub>q</sub> Bn); HRMS: [C<sub>91</sub>H<sub>107</sub>F<sub>17</sub>N<sub>6</sub>O<sub>33</sub>P<sub>6</sub>S + 2H]<sup>2+</sup> requires 1177.2450, found 1177.2466.



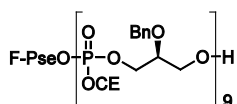
### 2-O-Benzyl-glycerol phosphate heptamer (13)

Glycerol phosphate hexamer **12** (238 mg, 101 μmol) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 1.15 ml, 202 μmol, 2 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Heptamer **13** (225 mg, 84.7 μmol, 84 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.9 (1P), -1.4 - -1.1 (5P), -0.9, -0.9 (1P); <sup>1</sup>H NMR (400 MHz): δ = 2.10 - 2.36 (m, 5H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub>OH), 2.52 - 2.69 (m, 14H, 7 x CH<sub>2</sub> cyanoethyl), 3.06 - 3.13 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.25 - 3.34 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.64 - 3.71 (m, 3H, CH glycerol, CH<sub>2</sub> glycerol), 3.78 - 3.85 (m, 6H, 6 x CH glycerol), 4.07 - 4.34 (m, 40H, 13 x CH<sub>2</sub> glycerol, 7 x CH<sub>2</sub> cyanoethyl), 4.43 - 4.50 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 4.58 - 4.67 (m, 14H, 7 x CH<sub>2</sub> Bn), 7.26 - 7.36 (m, 35H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 13.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 19.3 - 19.5 (7 x CH<sub>2</sub> cyanoethyl), 29.3 (t, J = 22 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 53.0 - 53.2 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 60.4 - 60.5 (CH<sub>2</sub> glycerol), 61.3 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 62.0 - 62.4 (7 x CH<sub>2</sub> cyanoethyl), 65.4 - 66.6 (13 x CH<sub>2</sub> glycerol), 71.9 (CH<sub>2</sub> Bn), 72.1 (6 x CH<sub>2</sub> Bn), 75.2 - 75.5 (6 x CH glycerol), 77.5 - 77.6 (CH glycerol), 116.6 - 116.8 (7 x C<sub>q</sub> cyanoethyl), 127.8 - 128.5 (CH<sub>arom</sub>), 137.1 - 137.3 (6 x C<sub>q</sub> Bn), 137.7 (C<sub>q</sub> Bn); HRMS: [C<sub>104</sub>H<sub>123</sub>F<sub>17</sub>N<sub>7</sub>O<sub>38</sub>P<sub>7</sub>S + 2Na]<sup>2+</sup> requires 1348.2669, found 1348.2666.



### 2-O-Benzyl-glycerol phosphate octamer (14)

Glycerol phosphate heptamer **13** (222 mg, 83.7 μmol) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 0.96 ml, 167 μmol, 2 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Octamer **14** (206 mg, 69.7 μmol, 83 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.8 (1P), -1.4 - -1.1 (6P), -0.9 (1P); <sup>1</sup>H NMR (400 MHz): δ = 2.10 - 2.36 (m, 5H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub>OH), 2.51 - 2.68 (m, 16H, 8 x CH<sub>2</sub> cyanoethyl), 3.06 - 3.13 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.25 - 3.33 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.64 - 3.72 (m, 3H, CH glycerol, CH<sub>2</sub> glycerol), 3.78 - 3.85 (m, 7H, 7 x CH glycerol), 4.07 - 4.33 (m, 46H, 15 x CH<sub>2</sub> glycerol, 8 x CH<sub>2</sub> cyanoethyl), 4.43 - 4.50 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 4.57 - 4.66 (m, 16H, 8 x CH<sub>2</sub> Bn), 7.26 - 7.37 (m, 40H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 13.3 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 19.3 - 19.4 (8 x CH<sub>2</sub> cyanoethyl), 29.3 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 53.0 - 53.2 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 60.4 - 60.5 (CH<sub>2</sub> glycerol), 61.3 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 62.0 - 62.4 (8 x CH<sub>2</sub> cyanoethyl), 65.4 - 66.6 (15 x CH<sub>2</sub> glycerol), 71.9 (CH<sub>2</sub> Bn), 72.1 (7 x CH<sub>2</sub> Bn), 75.2 - 75.5 (7 x CH glycerol), 77.5 - 77.6 (CH glycerol), 116.6 - 116.7 (8 x C<sub>q</sub> cyanoethyl), 127.8 - 128.5 (CH<sub>arom</sub>), 137.2 - 137.3 (7 x C<sub>q</sub> Bn), 137.8 (C<sub>q</sub> Bn); HRMS: [C<sub>117</sub>H<sub>139</sub>F<sub>17</sub>N<sub>8</sub>O<sub>43</sub>P<sub>8</sub>S + 2Na]<sup>2+</sup> requires 1496.8052, found 1496.8054.

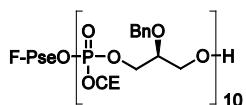


### 2-O-Benzyl-glycerol phosphate nonamer (15)

Glycerol phosphate octamer **14** (200 mg, 68.0 μmol) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 0.78 ml, 136 μmol, 2 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Nonamer **15** (182 mg, 56.1 μmol, 83 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.9 (1P), -1.3 - -1.1 (7P), -0.9 (1P); <sup>1</sup>H NMR (400 MHz): δ = 2.11 - 2.41 (m, 5H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub>OH), 2.51 - 2.70 (m, 18H, 9 x CH<sub>2</sub> cyanoethyl), 3.06 - 3.14 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.25

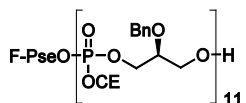


- 3.34 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.64 - 3.71 (m, 3H, CH glycerol, CH<sub>2</sub> glycerol), 3.78 - 3.85 (m, 8H, 8 x CH glycerol), 4.07 - 4.33 (m, 52H, 17 x CH<sub>2</sub> glycerol, 9 x CH<sub>2</sub> cyanoethyl), 4.43 - 4.50 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 4.58 - 4.66 (m, 18H, 9 x CH<sub>2</sub> Bn), 7.26 - 7.37 (m, 45H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 13.3 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 19.3 - 19.4 (9 x CH<sub>2</sub> cyanoethyl), 29.3 (t, J = 21 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 53.0 - 53.2 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 60.4 - 60.5 (CH<sub>2</sub> glycerol), 61.3 - 61.4 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 62.0 - 62.4 (9 x CH<sub>2</sub> cyanoethyl), 65.4 - 66.6 (17 x CH<sub>2</sub> glycerol), 71.9 (CH<sub>2</sub> Bn), 72.1 (8 x CH<sub>2</sub> Bn), 75.2 - 75.5 (8 x CH glycerol), 77.5 - 77.6 (CH glycerol), 116.6 - 116.8 (9 x C<sub>q</sub> cyanoethyl), 127.8 - 128.5 (CH<sub>arom</sub>), 137.2 - 137.3 (8 x C<sub>q</sub> Bn), 137.8 (C<sub>q</sub> Bn); HRMS: [C<sub>130</sub>H<sub>155</sub>F<sub>17</sub>N<sub>9</sub>O<sub>48</sub>P<sub>9</sub>S + 2Na]<sup>2+</sup> requires 1645.3435, found 1645.3433.



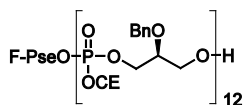
### 2-O-Benzyl-glycerol phosphate decamer (16)

Glycerol phosphate nonamer **15** (175 mg, 54.0 μmol) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 0.93 ml, 162 μmol, 3 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Decamer **16** (166 mg, 46.9 μmol, 87 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.9 (1P), -1.4 - -1.1 (8P), -0.9 (1P); <sup>1</sup>H NMR (400 MHz): δ = 2.10 - 2.40 (m, 5H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub>OH), 2.51 - 2.69 (m, 20H, 10 x CH<sub>2</sub> cyanoethyl), 3.06 - 3.14 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.25 - 3.34 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.64 - 3.71 (m, 3H, CH glycerol, CH<sub>2</sub> glycerol), 3.78 - 3.86 (m, 9H, 9 x CH glycerol), 4.06 - 4.34 (m, 58H, 19 x CH<sub>2</sub> glycerol, 10 x CH<sub>2</sub> cyanoethyl), 4.43 - 4.49 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 4.57 - 4.65 (m, 20H, 10 x CH<sub>2</sub> Bn), 7.25 - 7.37 (m, 50H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 13.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 19.3 - 19.5 (10 x CH<sub>2</sub> cyanoethyl), 29.3 (t, J = 21 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 53.0 - 53.2 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 60.4 - 60.5 (CH<sub>2</sub> glycerol), 61.3 - 61.4 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 62.0 - 62.4 (10 x CH<sub>2</sub> cyanoethyl), 65.4 - 66.6 (19 x CH<sub>2</sub> glycerol), 71.9 (CH<sub>2</sub> Bn), 72.1 (9 x CH<sub>2</sub> Bn), 75.2 - 75.5 (9 x CH glycerol), 77.5 - 77.6 (CH glycerol), 116.7 - 116.8 (10 x C<sub>q</sub> cyanoethyl), 127.8 - 128.5 (CH<sub>arom</sub>), 137.2 - 137.3 (9 x C<sub>q</sub> Bn), 137.7 (C<sub>q</sub> Bn); HRMS: [C<sub>143</sub>H<sub>171</sub>F<sub>17</sub>N<sub>10</sub>O<sub>53</sub>P<sub>10</sub>S + 2Na]<sup>2+</sup> requires 1793.8818, found 1793.8812.



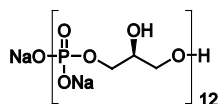
### 2-O-Benzyl-glycerol phosphate undecamer (17)

Glycerol phosphate decamer **16** (165 mg, 46.6 μmol) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 0.80 ml, 140 μmol, 3 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Undecamer **17** (159 mg, 41.5 μmol, 89 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.9 (1P), -1.4 - -1.1 (9P), -0.9, -0.9 (1P); <sup>1</sup>H NMR (400 MHz): δ = 2.10 - 2.37 (m, 4H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub>OH), 2.46 - 2.68 (m, 23H, 11 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>OH), 3.06 - 3.13 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.25 - 3.34 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.64 - 3.70 (m, 3H, CH glycerol, CH<sub>2</sub> glycerol), 3.77 - 3.85 (m, 10H, 10 x CH glycerol), 4.06 - 4.31 (m, 64H, 21 x CH<sub>2</sub> glycerol, 11 x CH<sub>2</sub> cyanoethyl), 4.43 - 4.50 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 4.57 - 4.66 (m, 22H, 11 x CH<sub>2</sub> Bn), 7.26 - 7.36 (m, 55H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 13.3 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 19.2 - 19.4 (11 x CH<sub>2</sub> cyanoethyl), 29.3 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 53.0 - 53.1 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 60.3 - 60.4 (CH<sub>2</sub> glycerol), 61.3 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 62.0 - 62.4 (11 x CH<sub>2</sub> cyanoethyl), 65.4 - 66.6 (21 x CH<sub>2</sub> glycerol), 71.9 (CH<sub>2</sub> Bn), 72.1 (10 x CH<sub>2</sub> Bn), 75.2 - 75.5 (10 x CH glycerol), 77.5 - 77.6 (CH glycerol), 116.6 - 116.8 (11 x C<sub>q</sub> cyanoethyl), 127.8 - 128.5 (CH<sub>arom</sub>), 137.2 - 137.3 (10 x C<sub>q</sub> Bn), 137.7 (C<sub>q</sub> Bn); HRMS: [C<sub>156</sub>H<sub>187</sub>F<sub>17</sub>N<sub>11</sub>O<sub>58</sub>P<sub>11</sub>S + 2H]<sup>2+</sup> requires 1920.4382, found 1920.4386.



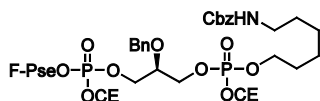
### 2-O-Benzyl-glycerol phosphate dodecamer (18)

Glycerol phosphate undecamer **17** (159 mg, 41.4  $\mu\text{mol}$ ) was coupled to glycerol phosphoramidite **6** (0.175 M in MeCN, 0.95 ml, 166  $\mu\text{mol}$ , 4 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Dodecamer **18** (161 mg, 38.8  $\mu\text{mol}$ , 94 %) was obtained as a colorless oil.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta$  = -1.9, -1.9 (1P), -1.4 - -1.1 (10P), -0.9, -0.9, -0.9 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 2.11 - 2.40 (m, 5H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{CH}_2\text{OH}$ ), 2.52 - 2.69 (m, 24H, 12 x  $\text{CH}_2$  cyanoethyl), 3.06 - 3.13 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 3.25 - 3.34 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 3.64 - 3.71 (m, 3H, CH glycerol,  $\text{CH}_2$  glycerol), 3.78 - 3.85 (m, 11H, 11 x CH glycerol), 4.06 - 4.32 (m, 70H, 23 x  $\text{CH}_2$  glycerol, 12 x  $\text{CH}_2$  cyanoethyl), 4.43 - 4.50 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 4.57 - 4.65 (m, 24H, 12 x  $\text{CH}_2$  Bn), 7.26 - 7.37 (m, 60H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 13.3 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 19.2 - 19.5 (12 x  $\text{CH}_2$  cyanoethyl), 29.3 (t,  $J$  = 21 Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 53.0 - 53.2 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 60.4 - 60.5 ( $\text{CH}_2$  glycerol), 61.3 ( $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 62.0 - 62.4 (12 x  $\text{CH}_2$  cyanoethyl), 65.4 - 66.6 (23 x  $\text{CH}_2$  glycerol), 71.9 ( $\text{CH}_2$  Bn), 72.1 (11 x  $\text{CH}_2$  Bn), 75.2 - 75.5 (11 x CH glycerol), 77.5 - 77.6 (CH glycerol), 116.6 - 116.8 (12 x  $\text{C}_q$  cyanoethyl), 127.8 - 128.5 ( $\text{CH}_{\text{arom}}$ ), 137.1 - 137.3 (11 x  $\text{C}_q$  Bn), 137.7 ( $\text{C}_q$  Bn); HRMS:  $[\text{C}_{169}\text{H}_{203}\text{F}_{17}\text{N}_{12}\text{O}_{63}\text{P}_{12}\text{S} + 3\text{Na}]^{3+}$  requires 1401.6354, found 1401.6361.



### Glycerol phosphate dodecamer (19)

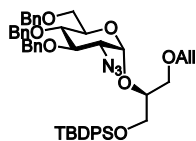
Protected dodecamer **18** (72.4 mg, 17.5  $\mu\text{mol}$ ) was treated with aqueous ammonia as described above. The compound was eluted ( $\text{H}_2\text{O}$ , ~5 ml) through a small column containing Dowex  $\text{Na}^+$  cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in  $\text{H}_2\text{O}$ , flushed with MeOH and  $\text{H}_2\text{O}$  until pH neutral before use) and, subsequently, lyophilized, yielding the intermediate dodecamer (56.5 mg, 17.5  $\mu\text{mol}$ , 100 %) as an amorphous white solid. Analytical data intermediate:  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.0 - 1.2 (11P), 2.5 (1P, phosphomonoester);  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 3.46 - 3.71 (m, 14H, 12 x CH glycerol,  $\text{CH}_2$  glycerol), 3.72 - 4.01 (m, 46H, 23 x  $\text{CH}_2$  glycerol), 4.26 - 4.58 (m, 24H, 12 x  $\text{CH}_2$  Bn), 6.93 - 7.33 (m, 60H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 60.2 ( $\text{CH}_2$  glycerol), 63.2 ( $\text{CH}_2$  glycerol), 64.3 - 64.6 (22 x  $\text{CH}_2$  glycerol), 71.6 ( $\text{CH}_2$  Bn), 71.8 - 72.0 (11 x  $\text{CH}_2$  Bn), 76.9 - 77.1 (11 x CH glycerol), 78.1 (CH glycerol), 127.9 - 128.6 ( $\text{CH}_{\text{arom}}$ ), 137.4 - 137.6 (12 x  $\text{C}_q$  Bn); HRMS:  $[\text{C}_{120}\text{H}_{158}\text{O}_{61}\text{P}_{12} + 2\text{NH}_4]^{2+}$  requires 1491.8411, found 1491.8423. A portion of the intermediate (53.0 mg, 16.4  $\mu\text{mol}$ ) was deprotected with Pd (0)/ $\text{H}_2$  using the standard procedure. Dodecaglycerolphosphate **19** (30.3 mg, 14.1  $\mu\text{mol}$ , 86 %) was obtained as an amorphous white solid.  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.2 - 1.3 (11P), 2.8 (1P, phosphomonoester);  $^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 3.59 (dd, 1H,  $J$  = 6.1 Hz, 11.8 Hz,  $\text{CHH}$  glycerol), 3.67 (dd, 1H,  $J$  = 4.3 Hz, 11.8 Hz,  $\text{CHH}$  glycerol), 3.81 - 3.97 (m, 48H, 2 x CH glycerol, 23 x  $\text{CH}_2$  glycerol), 3.99 - 4.06 (m, 10H, 10 x CH glycerol);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 63.1 ( $\text{CH}_2$  glycerol), 66.3 (d,  $J$  = 4.9 Hz,  $\text{CH}_2$  glycerol), 67.1 (d,  $J$  = 5.5 Hz,  $\text{CH}_2$  glycerol), 67.2 (d,  $J$  = 5.4 Hz, 19 x  $\text{CH}_2$  glycerol), 67.4 (d,  $J$  = 5.8 Hz,  $\text{CH}_2$  glycerol), 67.4 (d,  $J$  = 5.7 Hz,  $\text{CH}_2$  glycerol), 70.5 (t,  $J$  = 8.0 Hz, 10 x CH glycerol), 70.9 (t,  $J$  = 7.7 Hz, CH glycerol), 71.7 (d,  $J$  = 7.8 Hz, CH glycerol); HRMS:  $\text{C}_{36}\text{H}_{86}\text{O}_{61}\text{P}_{12} + \text{NH}_4^+$  requires 1884.0817, found 1884.0826.



### 1-[(Perfluorooctylpropylsulfonyl)ethyl]-(2-cyanoethyl)-phosphate]-2-O-benzyl-3-O-[(6-Cbz-aminohexyl)-(2-cyanoethyl) phosphate]-sn-glycerol (21)

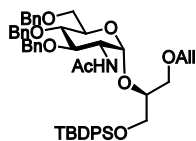
Monomeric glycerol phosphate alcohol **7** (182 mg, 209  $\mu\text{mol}$ ) was coupled to spacer phosphoramidite **20** (0.2 M in MeCN, 2.61 ml, 523  $\mu\text{mol}$ , 2.5 eq), oxidized and purified (F-SPE) as described in the general procedure. Construct **21** (243 mg, 197  $\mu\text{mol}$ , 94 %) was obtained as a colorless oil.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta$

= -1.8, -1.8 (1P), -1.1, -1.0 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.31 - 1.42 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.46 - 1.55 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.64 - 1.72 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.12 - 2.37 (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 2.63 - 2.72 (m, 4H, 2 x  $\text{CH}_2$  cyanoethyl), 3.07 - 3.21 (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{CH}_2\text{-N}$  hexylspacer), 3.25 - 3.36 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 3.85 - 3.91 (m, 1H, CH glycerol), 4.05 - 4.10 (m, 2H,  $\text{CH}_2\text{-O}$  hexylspacer), 4.14 - 4.29 (m, 7H,  $\text{CH}_2$  glycerol, CHH glycerol, 2 x  $\text{CH}_2$  cyanoethyl), 4.32 - 4.39 (m, 1H, CHH glycerol), 4.46 - 4.52 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 4.67 (s, 2H,  $\text{CH}_2$  Bn), 4.95 (bs, 1H, NH CBz), 5.09 (s, 2H,  $\text{CH}_2$  CBz), 7.29 - 7.38 (m, 10H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 13.3 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 19.4 - 19.5 (2 x  $\text{CH}_2$  cyanoethyl), 24.8, 25.9 (2 x  $\text{CH}_2$  hexylspacer), 29.2 (t,  $J$  = 23 Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 29.6 ( $\text{CH}_2$  hexylspacer), 29.8 (d,  $J$  = 6.6 Hz,  $\text{CH}_2$  hexylspacer), 40.7 ( $\text{CH}_2\text{-N}$  hexylspacer), 53.0 - 53.2 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 61.3 ( $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 61.8 - 61.9 ( $\text{CH}_2$  cyanoethyl), 62.3 - 62.4 ( $\text{CH}_2$  cyanoethyl), 65.2 - 65.3 ( $\text{CH}_2$  glycerol), 66.1 - 66.2 ( $\text{CH}_2$  glycerol), 66.4 ( $\text{CH}_2$  CBz), 68.3 - 68.5 ( $\text{CH}_2\text{-O}$  hexylspacer), 72.2 ( $\text{CH}_2$  Bn), 75.3 (t,  $J$  = 6.8 Hz, CH glycerol), 116.6 (2 x  $\text{C}_q$  cyanoethyl), 127.8 - 128.5 ( $\text{CH}_{\text{arom}}$ ), 136.6, 137.1 ( $\text{C}_q$  Bn,  $\text{C}_q$  CBz), 156.3 ( $\text{C=O}$  CBz); HRMS:  $\text{C}_{43}\text{H}_{50}\text{F}_{17}\text{N}_3\text{O}_{13}\text{P}_2\text{S} + \text{H}^+$  requires 1234.2341, found 1234.2342.



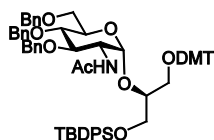
**1-*O*-tert-Butyldiphenylsilyl-2-*O*-(2-azido-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-3-*O*-allyl-*sn*-glycerol (26).**

To a cooled (0 °C) solution of triol **25** (5.09 g, 9.13 mmol) and benzyl bromide (10.2 ml, 85.0 mmol) in a 3/2 mixture of DMF/THF (110 ml) was added NaH (60 % dispersion in mineral oil, 2.90 g, 72.5 mmol). After stirring for 30 h at RT, MeOH (10 ml) was added and the mixture was allowed to stir for another 30 min. The volatiles were removed under reduced pressure and the residue was taken up in  $\text{Et}_2\text{O}$  (500 ml) and washed with  $\text{H}_2\text{O}$  (100 ml) and brine (300 ml). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo*, after which purification of the residue by column chromatography ( $\text{EtOAc/PE}$ ) yielded fully protected **26** (4.24 g, 5.12 mmol, 56%) as a colorless oil. Analytical data were in accordance to literature data.<sup>27</sup>



**1-*O*-tert-Butyldiphenylsilyl-2-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-3-*O*-allyl-*sn*-glycerol (27a).**

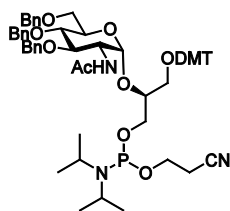
To a solution of compound **26** (3.38 g, 4.09 mmol) in a 5/1 mixture of 1,4-dioxane/ $\text{H}_2\text{O}$  (24 ml) was added  $\text{PMe}_3$  (1M in toluene, 8.2 ml, 8.2 mmol). After stirring for 4 h, the volatiles were removed under reduced pressure and, subsequently, the residue was coevaporated with toluene (3 x 20 ml). The residue was taken up in pyridine (20 ml) and, after the addition of  $\text{Ac}_2\text{O}$  (0.80 ml, 8.5 mmol), stirred overnight. The mixture was diluted with  $\text{Et}_2\text{O}$  (200 ml) and washed with  $\text{H}_2\text{O}$  (70 ml), sat. aq.  $\text{NaHCO}_3$  (70 ml) and brine (70 ml). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo* after which purification of the residue by column chromatography ( $\text{EtOAc/PE}$ ) gave title compound **27a** (2.80 g, 3.32 mmol, 81%) as a colourless oil. Analytical data were in accordance to literature data.<sup>27</sup>



**1-*O*-tert-Butyldiphenylsilyl-2-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-*sn*-glycerol (28a).**

To a solution of allyl ether **27a** (1.92 g, 2.11 mmol) in THF (29 ml) was added  $\text{Ir}(\text{COD})(\text{PPh}_2\text{Me})_2\text{PF}_6$  (89 mg, 0.11 mmol). The solution was shortly purged with  $\text{H}_2$  (g) (~10s) and stirred for 45 min under argon atmosphere. The mixture was diluted with sat. aq.  $\text{NaHCO}_3$  (10 ml) and  $\text{I}_2$  (1.45 g, 5.71 mmol) was added. After stirring for 30 min the mixture was diluted with  $\text{EtOAc}$  (250 ml) and washed

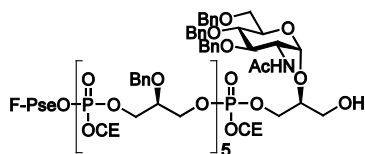
with sat. aq.  $\text{Na}_2\text{S}_2\text{O}_3$  (50 ml) and brine (100 ml), respectively. The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and the volatiles removed under reduced pressure after which purification of the residue by column chromatography (EtOAc/PE) gave the intermediate **1-*O*-tert-Butyldiphenylsilyl-2-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-sn-glycerol** as a slightly yellow oil. Analytical data were in accordance to literature data.<sup>27</sup> To a cooled (0 °C) solution of 1-*O*-tert-butyldiphenylsilyl-2-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-sn-glycerol (2.17 g, 2.70 mmol) and  $\text{Et}_3\text{N}$  (0.60 ml, 4.33 mmol) in DCM (14 ml) was added DMT-Cl (1.17 g, 3.46 mmol). The mixture was stirred for 2 hrs before MeOH (5.0 ml) was added. After stirring for an additional 15 minutes the reaction mixture was diluted with DCM (40 ml) and washed with a 1/1 mixture of sat. aq.  $\text{NaHCO}_3$  and brine (30 ml). The aqueous layer was extracted with DCM (2 x 10 ml) and the combined organic layers were dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure. The residual oil was purified by silica gel column chromatography (EtOAc/PE, containing ~0.5%  $\text{Et}_3\text{N}$ ) yielding DMT ether **28a** (2.57 g, 2.32 mmol, 86%) as an off white oil.  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): +41.0; IR (neat): 1028, 1248, 1508, 1684, 2906, 2930;  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 0.95 (s, 9H, *t*-Bu TBDPS), 1.53 (s, 3H, NHAc), 3.20 (dd, 1H,  $J$  = 6.4 Hz, 10.1 Hz, CHH glycerol), 3.36 (d, 1H,  $J$  = 10.2 Hz, H-6), 3.40 (dd, 1H,  $J$  = 3.5 Hz, 10.2 Hz, CHH glycerol), 3.59 (dd, 1H,  $J$  = 2.5 Hz, 10.7 Hz, H-6'), 3.63 - 3.67 (m, 2H, H-3, CHH glycerol), 3.72 - 3.82 (m, 9H, H-4, H-5, CHH glycerol, 2 x OMe), 3.89 - 3.94 (m, 1H, CH glycerol), 4.29 - 4.39 (m, 2H, H-2, CHH Bn), 4.47 (d, 1H,  $J$  = 10.9 Hz, CHH Bn), 4.56 (d, 1H,  $J$  = 12.2 Hz, CHH Bn), 4.61 (d, 1H,  $J$  = 11.5 Hz, CHH Bn), 4.77 (d, 1H,  $J$  = 10.8 Hz, CHH Bn), 4.79 (d, 1H,  $J$  = 11.4 Hz, CHH Bn), 4.96 (d, 1H,  $J$  = 3.7 Hz, H-1), 5.49 (d, 1H,  $J$  = 9.7 Hz, NH), 6.78 - 6.82 (m, 4H,  $\text{H}_{\text{arom}}$ ), 7.12 - 7.40 (m, 30H,  $\text{H}_{\text{arom}}$ ), 7.54 - 7.57 (m, 4H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 19.1 ( $\text{C}_q$  *t*-Bu), 23.3 ( $\text{CH}_3$  NHAc), 26.7 (3 x  $\text{CH}_3$  TBDPS), 52.4 (C-2), 55.2 (2 x OMe), 63.0 ( $\text{CH}_2$  glycerol), 63.6 ( $\text{CH}_2$  glycerol), 68.1 (C-6), 71.1 (C-5), 73.3 ( $\text{CH}_2$  Bn), 74.8 ( $\text{CH}_2$  Bn), 74.9 ( $\text{CH}_2$  Bn), 77.5 (CH glycerol), 78.0 (C-4), 81.0 (C-3), 86.2 ( $\text{C}_q$  DMTr), 97.6 (C-1), 113.1 ( $\text{CH}_{\text{arom}}$ ), 126.8 - 130.0 ( $\text{CH}_{\text{arom}}$ ), 133.1, 133.1 (2 x  $\text{C}_q$  phenyl), 135.4 ( $\text{CH}_{\text{arom}}$ ), 135.8, 135.9, 138.0, 138.2, 138.4 (2 x  $\text{C}_q$  DMTr, 3 x  $\text{C}_q$  Bn), 144.8 ( $\text{C}_q$  DMTr), 158.5 ( $\text{C}_q$  DMTr), 169.7 (C=O NHAc); HRMS:  $\text{C}_{69}\text{H}_{75}\text{NO}_{10}\text{Si}$  +  $\text{Na}^+$  requires 1128.5052, found 1128.5062.



**1-([*N,N*-Diisopropylamino]-2-cyanoethylphosphite)-2-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-sn-glycerol (29a)**

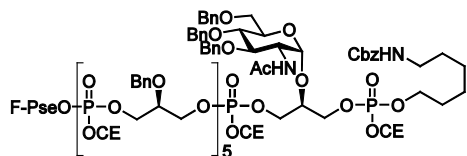
Compound **28a** (2.36 g, 2.13 mmol) was dissolved in THF (21 ml) and after addition of TBAF (1.00 M in THF, 3.20 ml, 3.20 mmol) stirred overnight. After evaporation of the solvents under reduced pressure the resulting oil was purified by column chromatography (EtOAc/PE, containing ~0.5%  $\text{Et}_3\text{N}$ ) giving the intermediate alcohol (1.74 g, 2.00 mmol, 94%) as a colourless oil. Analytical data **2-*O*-(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-sn-glycerol**:  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): +45.5; IR (neat): 906, 1373, 1541, 1663, 2900;  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.59 (s, 3H, NHAc), 3.18 (dd, 1H,  $J$  = 3.7 Hz, 10.2 Hz, CHH glycerol), 3.29 (dd, 1H,  $J$  = 6.7 Hz, 10.1 Hz, CHH glycerol), 3.54 - 3.59 (m, 3H, H-4, H-6, CHH glycerol), 3.67 - 3.78 (m, 10H, H-3, H-6', CH glycerol, CHH glycerol, 2 x OMe), 4.03 - 4.07 (m, 1H, H-5), 4.33 (td, 1H,  $J$  = 3.8 Hz, 10.0 Hz, 10.0 Hz, H-2), 4.47 - 4.57 (m, 3H, 3 x CHH Bn), 4.65 (d, 1H,  $J$  = 11.4 Hz, CHH Bn), 4.80 (d, 1H,  $J$  = 10.9 Hz, CHH Bn), 4.83 (d, 1H,  $J$  = 11.4 Hz, CHH Bn), 4.90 (d, 1H,  $J$  = 3.8 Hz, H-1), 5.53 (d, 1H,  $J$  = 9.5 Hz, NH), 6.80 - 6.83 (m, 4H,  $\text{H}_{\text{arom}}$ ), 7.15 - 7.41 (m, 24H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 23.2 ( $\text{CH}_3$  NHAc), 52.5 (C-2), 55.1 (2 x OMe), 63.4 ( $\text{CH}_2$  glycerol), 63.6 ( $\text{CH}_2$  glycerol), 68.7 (C-6), 71.4 (C-5), 73.4 ( $\text{CH}_2$  Bn), 75.0 ( $\text{CH}_2$  Bn), 75.1 ( $\text{CH}_2$  Bn), 78.4 (C-4), 81.0, 81.4 (C-3, CH glycerol), 86.3 ( $\text{C}_q$  DMTr), 98.2 (C-1), 113.1 ( $\text{CH}_{\text{arom}}$ ), 126.9 - 129.9 ( $\text{CH}_{\text{arom}}$ ), 135.7, 137.5, 137.7, 138.2 (2 x  $\text{C}_q$  DMTr, 3 x  $\text{C}_q$  Bn), 144.6 ( $\text{C}_q$  DMTr), 158.5 ( $\text{C}_q$  DMTr), 169.8 (C=O NHAc); HRMS:  $\text{C}_{53}\text{H}_{57}\text{NO}_{10}$  +  $\text{Na}^+$  requires 890.3875, found

890.3882. To a cooled (0 °C) solution of the intermediate alcohol (828 mg, 0.950 mmol) and Et<sub>3</sub>N (200 µl, 1.44 mmol) in freshly distilled DCM (10 ml) was added 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (250 µl, 1.12 mmol). After stirring overnight, the reaction was quenched by the addition of H<sub>2</sub>O (2.0 ml), diluted with DCM (40 ml) and washed with H<sub>2</sub>O (20 ml) and brine (20 ml), respectively. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE, containing ~0.5% Et<sub>3</sub>N) gave phosphoramidite **29a** (760 mg, 0.710 mmol, 75%, mixture of diastereomers) as a white foam. IR (neat): 1248, 1508, 1607, 1676, 2870, 2928, 3279; <sup>31</sup>P NMR (161.7 MHz, CD<sub>3</sub>CN): δ = 148.9, 149.2 (diastereoisomers); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN): δ = 1.09 - 1.16 (m, 12H, 4 x CH<sub>3</sub> isopropylamino), 1.67, 1.68 (2s, 3H, NHAc, diastereoisomers), 2.48 - 2.54 (m, 2H, CH<sub>2</sub> cyanoethyl), 3.14 - 3.24 (m, 1H, CHH glycerol), 3.30 - 3.36 (m, 1H, CHH glycerol), 3.50 - 3.90 (m, 16H, H-3, H-4, H-6, H-6', CH<sub>2</sub> glycerol, 2 x OMe, CH<sub>2</sub> cyanoethyl, 2 x CH isopropylamino), 3.92 - 4.00 (m, 1H, CH glycerol), 4.02 - 4.16 (m, 2H, H-2, H-5), 4.52 - 4.61 (m, 3H, 3 x CHH Bn), 4.69 (d, 1H, *J* = 11.2 Hz, CHH Bn), 4.79 - 4.82 (m, 2H, 2 x CHH Bn), 4.91, 4.93 (2d, 1H, *J* = 3.5 Hz, H-1, diastereoisomers), 6.33, 6.36 (2d, 1H, *J* = 9.7 Hz, NH), 6.87 (d, 4H, *J* = 8.8 Hz, Harom), 7.21 - 7.47 (m, 24H, Harom); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>CN): δ = 20.9, 20.9 (CH<sub>2</sub> cyanoethyl), 23.4 (CH<sub>3</sub> NHAc), 24.9 - 25.2 (4 x CH<sub>3</sub> isopropylamino), 43.7, 43.8 (2 x CH isopropylamino), 53.7 (C-2), 55.9 (2 x OMe), 59.3, 59.5 (CH<sub>2</sub> cyanoethyl), 63.4 (CH<sub>2</sub> glycerol), 64.4 - 64.6 (CH<sub>2</sub> glycerol), 69.9, 69.9 (C-6), 71.9, 72.0 (C-5), 73.8, 73.9 (CH<sub>2</sub> Bn), 75.4, 75.5 (CH<sub>2</sub> Bn), 75.7 (CH<sub>2</sub> Bn), 76.7, 77.1 (2d, *J* = 8 Hz, CH glycerol, diastereoisomers), 79.5, 79.6 (C-4), 81.6 (C-3), 87.0 (C<sub>q</sub> DMTr), 97.7, 98.0 (C-1), 114.1 (CH<sub>arom</sub>), 118.2 (C<sub>q</sub> cyanoethyl), 127.7 - 130.9 (CH<sub>arom</sub>), 136.9, 139.5 - 139.9 (2 x C<sub>q</sub> DMTr, 3 x C<sub>q</sub> Bn), 146.2 (C<sub>q</sub> DMTr), 159.6 (C<sub>q</sub> DMTr), 170.4 (C=O NHAc); HRMS: C<sub>62</sub>H<sub>74</sub>N<sub>3</sub>O<sub>11</sub>P + Na<sup>+</sup> requires 1090.4953, found 1090.4953.



#### GlcNAc-2-*O*-benzylglycerol phosphate hexamer (**30a**)

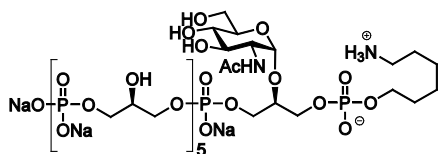
Glycerol phosphate pentamer **11** (74.1 mg, 36.0 µmol) and *N*-acetylglucosamineglycerol phosphoramidite **29a** (77.0 mg, 72.1 µmol, 2 eq) were dissolved in CH<sub>3</sub>CN (1.0 ml) together with freshly activated MS3Å and stirred for 15 min under argon. Subsequently, DCI (0.25M solution in CH<sub>3</sub>CN, 0.72 ml, 0.18 mmol) was added and the mixture stirred for 1 hr before water (0.50 ml) was added. The oxidation, detritylation and F-SPE steps were performed according to the general procedure. Hexamer **30a** (71.6 mg, 26.2 µmol, 73 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.8 (1P), -1.6 - -1.0 (5P); <sup>1</sup>H NMR (400 MHz): δ = 1.87 - 1.92 (bs, 3H, NHAc), 2.10 - 2.34 (m, 5H, CH<sub>2</sub>OH, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 2.42 - 2.70 (m, 12H, 6 x CH<sub>2</sub> cyanoethyl), 3.06 - 3.13 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.24 - 3.33 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.54 - 3.98 (m, 13H, H-3, H-4, H-5, H-6, H-6', 6 x CH glycerol, CH<sub>2</sub> glycerol), 4.03 - 4.34 (m, 35H, H-2, 11 x CH<sub>2</sub> glycerol, 6 x CH<sub>2</sub> cyanoethyl), 4.43 - 4.51 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 4.56 - 4.69 (m, 14H, 7 x CH<sub>2</sub> Bn), 4.75 - 4.80 (m, 2H, CH<sub>2</sub> Bn), 4.96 (m, 1H, H-1), 6.55 - 6.76 (m, 1H, NHAc), 7.11 - 7.17 (m, 2H, Harom), 7.22 - 7.36 (m, 38H, Harom); HRMS: [C<sub>113</sub>H<sub>132</sub>F<sub>17</sub>N<sub>7</sub>O<sub>38</sub>P<sub>6</sub>S + 2NH<sub>4</sub>]<sup>2+</sup> requires 1386.3598, found 1386.3608.



#### GlcNAc-2-*O*-benzylglycerol phosphate hexamer-aminohexyl spacer (**31a**)

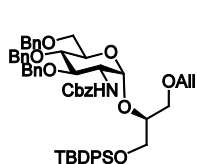
Hexamer **30a** (68.9 mg, 25.2 µmol) was coupled to phosphoramidite **20** (0.2 M in MeCN, 0.50 ml, 101 µmol, 4 eq), oxidized and purified (F-SPE) using the general procedure as described above. Oligomer **31a** (62.5 mg, 20.1 µmol, 80 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.8 (1P), -1.4 - -0.9

(5P), -0.8, -0.7 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.15 - 1.54 (m, 6H, 3 x  $\text{CH}_2$  hexylspacer), 1.63 - 1.69 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.96 - 2.00 (bs, 3H, NHAc), 2.06 - 2.36 (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ), 2.42 - 2.73 (m, 14H, 7 x  $\text{CH}_2$  cyanoethyl), 3.05 - 3.20 (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{CH}_2\text{-N}$  hexylspacer), 3.23 - 3.34 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ), 3.64 - 3.97 (m, 11H, H-3, H-4, H-5, H-6, H-6', 6 x CH glycerol), 4.03 - 4.38 (m, 41H, H-2, 12 x  $\text{CH}_2$  glycerol, 7 x  $\text{CH}_2$  cyanoethyl,  $\text{CH}_2\text{-O}$  hexylspacer), 4.42 - 4.51 (m, 4H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2^-$ ,  $\text{CH}_2$  Bn), 4.56 - 4.65 (m, 11H, 5 x  $\text{CH}_2$  Bn, CHH Bn), 4.68 - 4.80 (m, 3H,  $\text{CH}_2$  Bn, CHH Bn), 4.90 - 4.93 (m, 1H, H-1), 5.00 - 5.15 (m, 3H, NH CBz,  $\text{CH}_2$  CBz), 7.09 - 7.20 (m, 3H, NHAc,  $\text{H}_{\text{arom}}$ ), 7.22 - 7.38 (m, 43H,  $\text{H}_{\text{arom}}$ ); HRMS:  $[\text{C}_{130}\text{H}_{155}\text{F}_{17}\text{N}_9\text{O}_{43}\text{P}_7\text{S} + 2\text{NH}_4]^{2+}$  requires 1569.4271, found 1569.4276.



#### GlcNAc glycerol phosphate hexamer-aminoethyl spacer (32a)

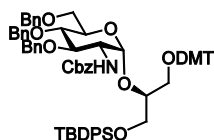
Protected hexamer **31a** (59.7 mg, 19.2  $\mu\text{mol}$ ) was treated with aqueous ammonia as described above. The compound was eluted through a small column containing Dowex  $\text{Na}^+$  cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in  $\text{H}_2\text{O}$ , flushed with  $\text{H}_2\text{O}$  and MeOH before use) and, subsequently, lyophilized, yielding the intermediate semi-protected hexamer (43.6 mg, 18.6  $\mu\text{mol}$ , 97 %) as an amorphous white solid. Analytical data intermediate:  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.0 - 1.3 (6P), 2.5 (1P, phosphomonoester);  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 0.90 - 1.30 (m, 6H, 3 x  $\text{CH}_2$  hexylspacer), 1.39 - 1.52 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.96 (bs, 3H, NHAc), 2.82 - 2.93 (m, 2H,  $\text{CH}_2\text{-N}$  hexylspacer), 3.43 - 4.17 (m, 40H, H-2, H-3, H-4, H-5, H-6, H-6', 12 x  $\text{CH}_2$  glycerol, 6 x CH glycerol,  $\text{CH}_2\text{-O}$  hexylspacer, 2 x CHH Bn), 4.28 - 4.55 (m, 14H, 6 x  $\text{CH}_2$  Bn, 2 x CHH Bn), 4.78 - 4.85 (m, 3H, NH CBz,  $\text{CH}_2$  CBz), 4.96 - 4.98 (m, 1H, H-1), 6.76 - 6.82 (m, 3H, NHAc,  $\text{H}_{\text{arom}}$ ), 6.96 - 7.29 (m, 43H,  $\text{H}_{\text{arom}}$ ); HRMS:  $[\text{C}_{96}\text{H}_{125}\text{N}_2\text{O}_{41}\text{P}_7 + 2\text{H}]^{2+}$  requires 1090.3033, found 1090.3043. A portion of the intermediate (43.0 mg, 18.4  $\mu\text{mol}$ ) was deprotected with Pd (0)/ $\text{H}_2$  using the standard procedure. monoGlcNAc hexamer **32a** (23.6 mg, 16.1  $\mu\text{mol}$ , 88 %) was obtained as an amorphous white solid.  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.0 (1P), 1.1 (1P), 1.3 (3P), 1.4 (1P), 3.2 (1P, phosphomonoester);  $^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.33 - 1.37 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.55 - 1.62 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.99 (s, 3H, NHAc), 2.92 (t, 2H,  $J$  = 7.5 Hz,  $\text{CH}_2\text{-N}$  hexylspacer), 3.39 (at, 1H,  $J$  = 9.6 Hz, H-3), 3.69 - 3.99 (m, 37H, H-2, H-4, H-5, H-6, H-6', 6 x CH glycerol, 12 x  $\text{CH}_2$  glycerol,  $\text{CH}_2\text{-O}$  hexylspacer), 5.00 (d, 1H,  $J$  = 3.5 Hz, H-1);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 23.0 ( $\text{CH}_3$  NHAc), 25.4, 26.1, 27.5 (3 x  $\text{CH}_2$  hexylspacer), 30.4 (d,  $J$  = 6.9 Hz,  $\text{CH}_2$  hexylspacer), 40.3 ( $\text{CH}_2\text{-N}$  hexylspacer), 54.6 (C-2), 61.4 (C-6), 65.2 (d,  $J$  = 5.1 Hz,  $\text{CH}_2$  glycerol), 66.0 - 66.1 ( $\text{CH}_2$  glycerol,  $\text{CH}_2\text{-O}$  hexylspacer), 67.0 - 67.4 (10 x  $\text{CH}_2$  glycerol), 70.4 (t,  $J$  = 7.9 Hz, 4 x CH glycerol), 70.9 - 71.0 (C-3, CH glycerol), 71.9 (C-4), 73.0 (C-5), 76.7 (t,  $J$  = 7.8 Hz, CH glycerol), 97.7 (C-1), 175.5 (C=O NHAc); HRMS:  $\text{C}_{32}\text{H}_{71}\text{N}_2\text{O}_{39}\text{P}_7 + \text{H}^+$  requires 1325.1870, found 1325.1873.



#### 1-*O*-*tert*-Butyldiphenylsilyl-2-*O*-(2-benzylcarbamate-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-3-*O*-allyl-*sn*-glycerol (27b)

To a solution of compound **26** (787 mg, 0.950 mmol) in dioxane/water (5/1, 6.0 ml) was added  $\text{PMe}_3$  (1.00 M in toluene, 2.00 ml, 2.00 mmol). This mixture was allowed to stir at rt for 4h after which the volatiles were removed *in vacuo*. The crude intermediate was, after coevaporation with toluene, redissolved in MeOH (7.0 ml) and  $\text{CHCl}_3$  (14 ml). To this mixture were added  $\text{NaHCO}_3$  (500 mg, 5.95 mmol) and benzylchloroformate (0.160 ml, 1.14 mmol). After stirring for 1.5 h, the solvents were removed under reduced pressure and the residue was taken up in EtOAc, filtrated and concentrated *in vacuo*. Purification by silica gel column chromatography

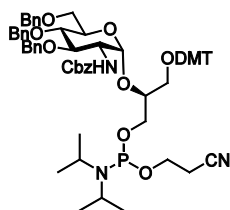
(EtOAc/PE) afforded carbamate **27b** (738 mg, 0.788 mmol, 83%) as a colorless oil.  $[\alpha]_D^{20}$  (CHCl<sub>3</sub>): +46.0; IR (neat): 908, 1024, 1045, 1508, 1719, 2858, 3030; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 1.03 (s, 9H, *t*-Bu TBDPS), 3.38 (ad, 1H, *J* = 10.7 Hz, H-6), 3.49 (m, 1H, CHH glycerol), 3.56 - 3.81 (m, 7H, H-4, H-5, H-6', CH glycerol, CHH glycerol, CH<sub>2</sub> glycerol), 3.86 - 4.04 (m, 4H, H-2, H-3, CH<sub>2</sub> allyl), 4.37 (d, 1H, *J* = 12.2 Hz, CHH Bn), 4.46 (d, 1H, *J* = 10.9 Hz, CHH Bn), 4.55 (d, 1H, *J* = 12.2 Hz, CHH Bn), 4.64 (d, 1H, *J* = 11.2 Hz, CHH Bn), 4.74 (d, 1H, *J* = 11.2 Hz, CHH Bn), 4.77 (d, 1H, *J* = 10.9 Hz, CHH Bn), 4.99 (d, 1H, *J* = 3.5 Hz, H-1), 5.02 - 5.08 (m, 2H, CHH Cbz, CHH allyl), 5.14 - 5.19 (m, 2H, CHH Cbz, CHH allyl), 5.31 (d, 1H, *J* = 9.7 Hz, NH), 5.79 (ddd, 1H, *J* = 5.6 Hz, 10.8 Hz, 16.2 Hz, CH allyl), 7.11 - 7.13 (m, 2H, H<sub>arom</sub>), 7.22 - 7.40 (m, 24H, H<sub>arom</sub>), 7.61 - 7.63 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 19.2 (C<sub>q</sub> *t*-Bu), 26.8 (3 x CH<sub>3</sub> TBDPS), 55.0 (C-2), 63.6 (CH<sub>2</sub> glycerol), 66.7 (CH<sub>2</sub> Cbz), 68.1 (C-6), 69.5 (CH<sub>2</sub> glycerol), 71.2 (C-5), 72.2 (CH<sub>2</sub> allyl), 73.3 (CH<sub>2</sub> Bn), 74.7 (CH<sub>2</sub> Bn), 75.2 (CH<sub>2</sub> Bn), 77.8 (C-3, CH glycerol), 81.3 (C-4), 98.4 (C-1), 117.2 (CH<sub>2</sub> allyl), 127.4 - 128.4 (CH<sub>arom</sub>), 129.7 (CH<sub>arom</sub>), 133.1, (C<sub>q</sub> phenyl), 134.4 (CH allyl), 135.5 (CH<sub>arom</sub>), 136.5, 138.0, 138.3, 138.4 (4 x C<sub>q</sub> Bn), 156.0 (C=O, Cbz); HRMS: C<sub>57</sub>H<sub>65</sub>NO<sub>9</sub>Si + Na<sup>+</sup> requires 958.4324, found 958.4321.



**1-*O*-tert-Butyldiphenylsilyl-2-*O*-(2-benzylcarbamate-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-3-*O*-(4,4'-dimethoxytrityl)-sn-glycerol (**28b**)**

A solution of glycoside **27b** (1.08 g, 1.15 mmol) in freshly distilled THF (10 ml) was stirred under argon for 30 min. After the addition of Ir(COD)(Ph<sub>2</sub>MeP)<sub>2</sub>PF<sub>6</sub> (48 mg, 0.060 mmol) the solution was purged with H<sub>2</sub> (g) for ~15s. After stirring under argon for 1.5 hrs, the mixture was diluted with THF (10 ml) and sat. aq. NaHCO<sub>3</sub> (10 ml). Upon addition of I<sub>2</sub> (0.58 g, 2.3 mmol), the mixture was allowed to stir for 30 mins at room temperature. The mixture was then diluted with EtOAc (100 ml) and washed with sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (30 ml) and brine (40 ml), respectively. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Column chromatography (EtOAc/PE) afforded the intermediate alcohol (963 mg, 1.09 mmol, 95%) as a pale yellow oil. Analytical data **1-*O*-tert-butylidiphenylsilyl-2-*O*-(2-benzylcarbamate-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-sn-glycerol**:  $[\alpha]_D^{20}$  (CHCl<sub>3</sub>): +57.0; IR (neat): 816, 1036, 1452, 1549, 1670, 1697, 2924, 3065, 3306; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 1.03 (s, 9H, *t*-Bu TBDPS), 2.27 (bs, 1H, OH), 3.37 (ad, 1H, *J* = 10.2 Hz, H-6), 3.56 (ad, 1H, *J* = 10.0 Hz, H-6'), 3.64 - 3.84 (m, 8H, H-3, H-4, H-5, CH glycerol, 2 x CH<sub>2</sub> glycerol), 3.94 - 4.00 (m, 1H, H-2), 4.38 (d, 1H, *J* = 12.2 Hz, CHH Bn), 4.46 (d, 1H, *J* = 10.8 Hz, CHH Bn), 4.53 (d, 1H, *J* = 12.2 Hz, CHH Bn), 4.62 (d, 1H, *J* = 10.9 Hz, CHH Bn), 4.73 - 4.78 (m, 2H, 2 x CHH Bn), 4.99 - 5.05 (m, 2H, H-1, CHH Cbz), 5.14 (d, 1H, *J* = 12.2 Hz, CHH Cbz), 5.21 (d, 1H, *J* = 8.0 Hz, NH), 7.06 - 7.41 (m, 26H, H<sub>arom</sub>), 7.61 - 7.65 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 19.1 (C<sub>q</sub> *t*-Bu), 26.8 (3 x CH<sub>3</sub> TBDPS), 55.0 (C-2), 62.2 (CH<sub>2</sub> glycerol), 64.2 (CH<sub>2</sub> glycerol), 66.8 (CH<sub>2</sub> Cbz), 68.1 (C-6), 71.1 (C-5), 73.3 (CH<sub>2</sub> Bn), 74.7 (CH<sub>2</sub> Bn), 75.1 (CH<sub>2</sub> Bn), 77.9, 78.1, (C-3, CH glycerol), 80.7 (C-4), 97.4 (C-1), 127.6 - 128.4 (CH<sub>arom</sub>), 129.8 (CH<sub>arom</sub>), 132.8 (C<sub>q</sub> phenyl), 135.5 (CH<sub>arom</sub>), 136.4, 137.8, 138.2 (4 x C<sub>q</sub> Bn), 156.1 (C=O, Cbz); HRMS: C<sub>54</sub>H<sub>61</sub>NO<sub>9</sub>Si + Na<sup>+</sup> requires 918.4009, found 918.4008. To a cooled (0 °C) solution of the intermediate alcohol (755 mg, 0.858 mmol) and Et<sub>3</sub>N (0.19 ml, 1.3 mmol) in DCM (4.3 ml) was added DMTr-Cl (349 mg, 1.02 mmol). The mixture was stirred for 2 hrs before MeOH (1.0 ml) was added. After stirring for an additional 15 minutes the reaction mixture was diluted with DCM (40 ml) and washed with a 1/1 mixture of sat. aq. NaHCO<sub>3</sub> and brine (30 ml). The aqueous layer was extracted with DCM (2 x 10 ml) and the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The residual oil was purified by silica gel column chromatography (EtOAc/PE, containing ~0.5% Et<sub>3</sub>N) yielding DMTr-ether **28b** (962 mg, 0.803 mmol, 94%) as an off white oil.  $[\alpha]_D^{20}$  (CHCl<sub>3</sub>): +52.0; IR (neat): 1028, 1248, 2951, 1508, 1726, 2857, 2901, 2930; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 0.93 (s, 9H, *t*-Bu TBDPS), 3.10 (dd, 1H, *J* = 7.0 Hz, 10.3

Hz, CHH glycerol), 3.37 (ad, 1H,  $J$  = 10.6 Hz, H-6), 3.44 (dd, 1H,  $J$  = 2.1 Hz, 9.8 Hz, CHH glycerol), 3.55 - 3.63 (m, 3H, H-3, H-6', CHH glycerol), 3.71 - 3.78 (m, 9H, H-4, H-5, CHH glycerol, 2 x OMe), 3.96 - 4.01 (m, 1H, CH glycerol), 4.05 - 4.11 (m, 1H, H-2), 4.39 (d, 1H,  $J$  = 12.2 Hz, CHH Bn), 4.45 (d, 1H,  $J$  = 10.9 Hz, CHH Bn), 4.57 (d, 1H,  $J$  = 12.2 Hz, CHH Bn), 4.60 (d, 1H,  $J$  = 11.9 Hz, CHH Bn), 4.63 (d, 1H,  $J$  = 12.5 Hz, CHH Cbz), 4.72 (d, 1H,  $J$  = 11.2 Hz, CHH Bn), 4.76 (d, 1H,  $J$  = 10.9 Hz, CHH Bn), 4.93 (d, 1H,  $J$  = 9.9 Hz, NH), 5.09 (d, 1H,  $J$  = 12.0 Hz, CHH Cbz), 5.15 (d, 1H,  $J$  = 3.3 Hz, H-1), 6.70 - 6.83 (m, 4H,  $H_{arom}$ ), 7.11 - 7.41 (m, 35H,  $H_{arom}$ ), 7.51 - 7.56 (m, 4H,  $H_{arom}$ );  $^{13}C$  NMR (100 MHz):  $\delta$  = 19.1 ( $C_q$  *t*-Bu), 26.7 (3 x  $CH_3$  TBDPS), 54.8 (C-2), 55.1 (2 x OMe), 63.6 ( $CH_2$  glycerol), 63.8 ( $CH_2$  glycerol), 66.7 ( $CH_2$  Cbz), 68.1 (C-6), 71.1 (C-5), 73.3 ( $CH_2$  Bn), 74.8 ( $CH_2$  Bn), 75.2 ( $CH_2$  Bn), 77.4 (CH glycerol), 77.8 (C-4), 81.4 (C-3), 86.2 ( $C_q$  DMTr), 98.1 (C-1), 113.1 ( $CH_{arom}$ ), 126.6 - 130.0 ( $CH_{arom}$ ), 133.0, 133.1 (2 x  $C_q$  phenyl), 135.4 ( $CH_{arom}$ ), 135.6, 136.0, 136.3, 137.9, 138.2, 138.3 (2 x  $C_q$  DMTr, 4 x  $C_q$  Bn), 144.9 ( $C_q$  DMTr), 155.8 (C=O, Cbz), 158.3 ( $C_q$  DMTr); HRMS:  $C_{75}H_{79}NO_{11}Si$  +  $Na^+$  requires 1220.5320, found 1220.5315.

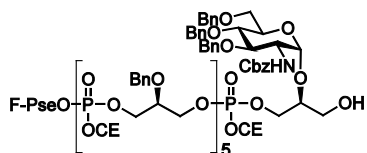


**1-([N,N]-Diisopropylamino)-2-cyanoethylphosphite-2-O-(2-benzylcarbamate-3,4,6-tri-O-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-sn-glycerol (29b)**

Compound **28b** (900 mg, 0.751 mmol) was dissolved in THF (7.50 ml) and after addition of TBAF (1.00 M in THF, 1.20 ml, 1.20 mmol) stirred overnight. After evaporation of the solvents under reduced pressure the resulting oil was purified by column chromatography (EtOAc/PE, containing ~0.5%  $Et_3N$ ), giving the intermediate alcohol (711 mg, 0.741 mmol, 99%) as a colourless oil. Analytical data **2-O-(2-Benzylcarbamate-3,4,6-tri-O-benzyl-2-deoxy- $\alpha$ -D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-sn-glycerol**:  $[\alpha]_D^{20}$  (CHCl<sub>3</sub>): +59.4; IR (neat): 906, 1209, 1714, 2249;  $^1H$  NMR (400 MHz):  $\delta$  = 2.79 (bs, 1H, OH), 3.20 - 3.23 (m, 2H,  $CH_2$  glycerol), 3.51 - 3.60 (m, 3H, H-4, H-6, CHH glycerol), 3.65 - 3.78 (m, 10H, H-3, H-6', CH glycerol, CHH glycerol, 2 x OMe), 4.00 - 4.11 (m, 2H, H-2, H-5), 4.47 (d, 1H,  $J$  = 10.9 Hz, CHH Bn), 4.51 (d, 1H,  $J$  = 12.3 Hz, CHH Bn), 4.57 (d, 1H,  $J$  = 12.3 Hz, CHH Bn), 4.65 (d, 1H,  $J$  = 11.1 Hz, CHH Bn), 4.75 - 4.81 (m, 3H, 2 x CHH Bn, CHH Cbz), 4.96 (d, 1H,  $J$  = 9.9 Hz, NH), 5.01 (d, 1H,  $J$  = 3.5 Hz, H-1), 5.12 (d, 1H,  $J$  = 12.2 Hz), CHH Cbz), 6.75 - 6.78 (m, 4H,  $H_{arom}$ ), 7.12 - 7.41 (m, 29H,  $H_{arom}$ );  $^{13}C$  NMR (100 MHz):  $\delta$  = 54.9 (C-2), 55.1 (2 x OMe), 63.5 (2 x  $CH_2$  glycerol), 66.8 ( $CH_2$  Cbz), 68.7 (C-6), 71.4 (C-5), 73.4 ( $CH_2$  Bn), 75.0 ( $CH_2$  Bn), 75.4 ( $CH_2$  Bn), 78.3 (C-4), 81.1 (CH glycerol), 81.5 (C-3), 86.3 ( $C_q$  DMTr), 98.5 (C-1), 113.1 ( $CH_{arom}$ ), 126.7 - 130.0 ( $CH_{arom}$ ), 135.7, 135.8, 136.3, 137.5, 137.8, 138.1 (2 x  $C_q$  DMTr, 4 x  $C_q$  Bn), 144.7 ( $C_q$  DMTr), 155.9 (C=O, Cbz), 158.4 ( $C_q$  DMTr); HRMS:  $C_{59}H_{61}NO_{11}$  +  $Na^+$  requires 982.4137, found 982.4142. To a cooled (0 °C) solution of the intermediate alcohol (969 mg, 1.01 mmol) and  $Et_3N$  (220  $\mu$ l, 1.52 mmol) in freshly distilled DCM (10 ml) was added 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (267  $\mu$ l, 1.20 mmol). After stirring overnight, the reaction was quenched by the addition of  $H_2O$  (2.0 ml), diluted with DCM (40 ml) and washed with, respectively,  $H_2O$  (20 ml) and brine (20 ml). The organic layer was dried over  $Na_2SO_4$  and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE, containing ~0.5%  $Et_3N$ ) gave phosphoramidite **29b** (939 mg, 0.810 mmol, 80%) as a white foam. IR (neat): 1248, 1508, 1606, 1724, 2837, 2870, 2930, 2965, 3063;  $^{31}P$  NMR (161.7 MHz,  $CD_3CN$ ):  $\delta$  = 149.1, 149.2 (diastereoisomers);  $^1H$  NMR (400 MHz,  $CD_3CN$ ):  $\delta$  = 1.03 - 1.12 (m, 12H, 4 x  $CH_3$  isopropylamino), 2.40 - 2.47 (m, 2H,  $CH_2$  cyanoethyl), 3.07 - 3.18 (m, 1H, CHH glycerol), 3.27 - 3.33 (m, 1H, CHH glycerol), 3.44 - 4.04 (m, 19H, H-2, H-3, H-4, H-5, H-6, H-6', CH glycerol,  $CH_2$  glycerol, 2 x OMe,  $CH_2$  cyanoethyl, 2 x CH isopropylamino), 4.50 - 4.80 (m, 7H, 3 x  $CH_2$  Bn, CHH Cbz), 4.99 - 5.07 (m, 2H, H-1, CHH Cbz), 5.43 - 5.49 (m, 1H, NH), 6.77 - 6.82 (m, 4H,  $H_{arom}$ ), 7.16 - 7.45 (m, 29H,  $H_{arom}$ );  $^{13}C$  NMR (100 MHz,  $CD_3CN$ ):  $\delta$  = 20.9, 20.9 ( $CH_2$  cyanoethyl), 24.8 - 25.1 (4 x  $CH_3$  isopropylamino), 43.7, 43.8 (2 x CH isopropylamino), 55.9 (2 x OMe), 56.2 (C-2), 59.3, 59.5 ( $CH_2$  cyanoethyl), 64.0 - 64.4 (2 x

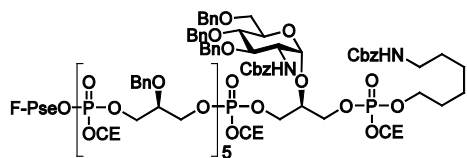


CH<sub>2</sub> glycerol), 67.1 (CH<sub>2</sub> Cbz), 69.8, 69.9 (C-6), 72.0 (C-5), 73.8, 73.9 (CH<sub>2</sub> Bn), 75.4, 75.4 (CH<sub>2</sub> Bn), 76.0 (CH<sub>2</sub> Bn), 77.3 - 77.5 (CH glycerol), 79.4 (C-4), 82.1 (C-3), 87.1 (C<sub>q</sub> DMTr), 98.6, 98.7 (C-1), 114.0 (CH<sub>arom</sub>), 118.3 (C<sub>q</sub> cyanoethyl), 127.7 - 131.0 (CH<sub>arom</sub>), 136.8, 137.0, 137.9, 139.5 - 139.7 (2 x C<sub>q</sub> DMTr, 4 x C<sub>q</sub> Bn), 146.2 (C<sub>q</sub> DMTr), 156.9 (C=O, Cbz), 159.5 (C<sub>q</sub> DMTr); HRMS: C<sub>68</sub>H<sub>78</sub>N<sub>3</sub>O<sub>12</sub>P + H<sup>+</sup> requires 1160.5396, found 1160.5392.



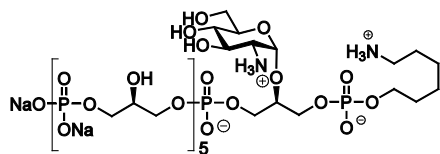
#### Cbz-glucosamine-2-O-benzylglycerol phosphate hexamer (30b)

Glycerol phosphate pentamer **11** (76.3 mg, 37.1 μmol) and *N*-Cbz-glucosamineglycerol phosphoramidite **29b** (86.1 mg, 74.2 μmol) were dissolved in CH<sub>3</sub>CN (1.0 ml) together with freshly activated MS3Å and stirred for 15 min under argon. Subsequently, DCI (0.25M solution in CH<sub>3</sub>CN, 0.4 ml, 0.19 mmol) was added and the mixture stirred for 1 hr before water (0.50 ml) was added. The oxidation, detritylation and F-SPE steps were performed according to the general procedure. Hexamer **30b** (91.4 mg, 32.5 μmol, 88 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.9 (1P), -1.4 - -1.0 (5P); <sup>1</sup>H NMR (400 MHz): δ = 2.09 - 2.69 (m, 17H, CH<sub>2</sub>OH, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, 6 x CH<sub>2</sub> cyanoethyl), 3.05 - 3.13 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.24 - 3.33 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.54 - 4.34 (m, 48H, H-2, H-3, H-4, H-5, H-6, H-6', 6 x CH glycerol, 12 x CH<sub>2</sub> glycerol, 6 x CH<sub>2</sub> cyanoethyl), 4.40 - 4.81 (m, 18H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, 8 x CH<sub>2</sub> Bn), 4.95 - 5.04 (m, 2H, H-1, CHH Cbz), 5.12 - 5.19 (m, 1H, CHH Cbz), 5.78 - 5.98 (m, 1H, NH Cbz), 7.10 - 7.15 (m, 2H, H<sub>arom</sub>), 7.22 - 7.38 (m, 43H, H<sub>arom</sub>); HRMS: [C<sub>119</sub>H<sub>136</sub>F<sub>17</sub>N<sub>7</sub>O<sub>39</sub>P<sub>6</sub>S + 2Na]<sup>2+</sup> requires 1437.3283, found 1437.3288.



#### Cbz-glucosamine-2-O-benzylglycerol phosphate hexamer aminohexyl spacer (31b)

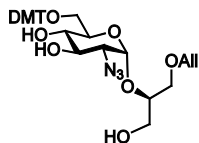
Hexamer **30b** (88.6 mg, 31.5 μmol) was coupled to spacer phosphoramidite **20** (0.2M in MeCN, 0.63 ml, 126 μmol, 4 eq), oxidized and purified (F-SPE) using the general procedure as described above. Hexamer **31b** (89.3 mg, 28.1 μmol, 89 %) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.8 (1P), -1.4 - -0.8 (6P); <sup>1</sup>H NMR (400 MHz): δ = 1.23 - 1.68 (m, 8H, 4 x CH<sub>2</sub> hexylspacer), 2.06 - 2.71 (m, 18H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, 7 x CH<sub>2</sub> cyanoethyl), 3.05 - 3.18 (m, 4H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub>-N hexylspacer), 3.23 - 3.34 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.67 - 3.88 (m, 11H, H-3, H-4, H-5, H-6, H-6', 6 x CH glycerol), 3.94 - 4.34 (m, 41H, H-2, 12 x CH<sub>2</sub> glycerol, 7 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>-O hexylspacer), 4.42 - 4.51 (m, 4H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub> Bn), 4.55 - 4.66 (m, 11H, 5 x CH<sub>2</sub> Bn, CHH Bn), 4.69 - 4.79 (m, 3H, CH<sub>2</sub> Bn, CHH Bn), 4.96 - 5.16 (m, 6H, H-1, NH Cbz, 2 x CH<sub>2</sub> Cbz), 5.93 - 6.23 (m, 1H, NH Cbz), 7.09 - 7.13 (m, 2H, H<sub>arom</sub>), 7.24 - 7.37 (m, 48H, H<sub>arom</sub>); HRMS: [C<sub>136</sub>H<sub>159</sub>F<sub>17</sub>N<sub>9</sub>O<sub>44</sub>P<sub>7</sub>S + 2Na]<sup>2+</sup> requires 1620.3956, found 1620.3957.



#### Glucosamine glycerol phosphate hexamer-aminohexyl spacer (32b)

Protected hexamer **31b** (87.3 mg, 27.5 μmol) was treated with aqueous ammonia as described above. The compound was eluted through a small column containing Dowex Na<sup>+</sup> cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in

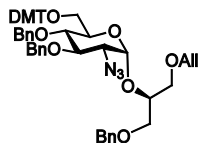
H<sub>2</sub>O, flushed with H<sub>2</sub>O and MeOH before use) and, subsequently, lyophilized, yielding the intermediate hexamer (66.3 mg, 27.5  $\mu$ mol, 100 %) as an amorphous white solid. Analytical data intermediate: <sup>31</sup>P NMR (161.7 MHz, D<sub>2</sub>O):  $\delta$  = 0.9 - 1.4 (6P), 3.4 (1P, phosphomonoester); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):  $\delta$  = 0.86 - 1.49 (m, 8H, 4 x CH<sub>2</sub> hexylspacer), 2.72 - 2.88 (m, 2H, CH<sub>2</sub>-N hexylspacer), 3.34 - 4.07 (m, 40H, H-2, H-3, H-4, H-5, H-6, H-6', 12 x CH<sub>2</sub> glycerol, 6 x CH glycerol, CH<sub>2</sub>-O hexylspacer, 2 x CHH Bn), 4.15 - 4.55 (m, 14H, 6 x CH<sub>2</sub> Bn, 2 x CHH Bn), 4.80 - 5.17 (m, 5H, H-1, 2 x CH<sub>2</sub> CBz), 6.64 - 6.69 (m, 2H, H<sub>arom</sub>), 6.75 - 7.29 (m, 48H, H<sub>arom</sub>); HRMS: [C<sub>102</sub>H<sub>129</sub>N<sub>2</sub>O<sub>42</sub>P<sub>7</sub> + 2NH<sub>4</sub>]<sup>2+</sup> requires 1153.8447, found 1153.8455. A portion of the intermediate (24.7 mg, 10.2  $\mu$ mol) was deprotected with Pd (0)/H<sub>2</sub> using the standard procedure. Glucosaminylated hexamer **32b** (11.7 mg, 8.33  $\mu$ mol, 81 %) was obtained as an amorphous white solid. <sup>31</sup>P NMR (161.7 MHz, D<sub>2</sub>O):  $\delta$  = 0.9 (1P), 1.2 (1P), 1.3 (2P), 1.4 (1P), 1.5 (1P), 2.6 (1P, phosphomonoester); <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O):  $\delta$  = 1.39 - 1.42 (m, 4H, 2 x CH<sub>2</sub> hexylspacer), 1.61 - 1.69 (m, 4H, 2 x CH<sub>2</sub> hexylspacer), 2.98 (t, 2H, *J* = 7.5 Hz, CH<sub>2</sub>-N hexylspacer), 3.32 (dd, 1H, *J* = 3.5 Hz, 10.6 Hz, H-2), 3.47 (at, 1H, *J* = 9.6 Hz, H-4), 3.75 - 4.08 (m, 35H, H-3, H-5, H-6, H-6', 5 x CH glycerol, 12 x CH<sub>2</sub> glycerol, CH<sub>2</sub>-O hexylspacer), 4.14 - 4.17 (m, 1H, CH glycerol), 5.40 (d, 1H, *J* = 3.5 Hz, H-1); <sup>13</sup>C NMR (150 MHz, D<sub>2</sub>O):  $\delta$  = 25.5, 26.1, 27.6 (3 x CH<sub>2</sub> hexylspacer), 30.4 (d, *J* = 6.7 Hz, CH<sub>2</sub> hexylspacer), 40.4 (CH<sub>2</sub>-N hexylspacer), 55.0 (C-2), 61.2 (C-6), 65.5 (d, *J* = 5.5 Hz, CH<sub>2</sub> glycerol), 65.9 (d, *J* = 5.3 Hz, CH<sub>2</sub> glycerol), 66.3 (d, *J* = 4.9 Hz, CH<sub>2</sub>-O hexylspacer), 67.1 - 67.5 (10 x CH<sub>2</sub> glycerol), 70.5 - 70.9 (C-4, C-5, 5 x CH glycerol), 73.3 (C-3), 76.5 (t, *J* = 7.7 Hz, CH glycerol), 95.7 (C-1); HRMS: C<sub>30</sub>H<sub>69</sub>N<sub>2</sub>O<sub>38</sub>P<sub>7</sub> + H<sup>+</sup> requires 1283.1764, found 1283.1769.



**3-O-Allyl-2-O-(2-azido-2-deoxy-6-O-[4,4'-dimethoxytrityl]-α-D-glucopyranosyl)-sn-glycerol (33)**

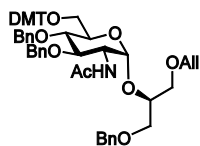
To a solution of glycoside **25** (1.26 g, 2.26 mmol) in DCM/1,4-dioxane (1/1, 60 ml) were added Et<sub>3</sub>N (0.490 ml, 3.39 mmol) and DMTr-Cl (0.920 g, 2.72 mmol), respectively. After stirring for 24h MeOH (10 ml) was added and the mixture stirred for 30 minutes after which the solution was further diluted with DCM (50 ml) and washed with water (10 ml) and brine (20 ml). The combined waterlayers were extracted with DCM (20 ml) and the organic layer was washed with brine. The combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*, after which purification with column chromatography (EtOAc/PE, containing ~0.5% Et<sub>3</sub>N) gave the intermediate diol as a pale yellow oil (1.51 g, 1.76 mmol, 78 %). Analytical data **3-O-allyl-2-O-(2-azido-2-deoxy-6-O-[4,4'-dimethoxytrityl]-α-D-glucopyranosyl)-1-O-tert-butylidiphenylsilyl-sn-glycerol**: [α]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +30.6; IR (neat): 1028, 1248, 1508, 1607, 2106, 2932; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 1.01 (s, 9H, *t*-Bu TBDPS), 3.16 (dd, 1H, *J* = 3.6 Hz, 10.4 Hz, H-2), 3.18 - 3.24 (m, 2H, H-6, H-6'), 3.59 - 3.65 (m, 2H, H-4, CHH glycerol), 3.69 - 3.84 (m, 10H, H-5, CH<sub>2</sub> glycerol, CHH glycerol, 2 x OMe), 3.95 - 4.02 (m, 4H, H-3, CH glycerol, CH<sub>2</sub> allyl), 5.18 (dd, 1H, *J* = 1.6 Hz, 10.4 Hz, CHH allyl), 5.25 - 5.30 (m, 2H, H-1, CHH allyl), 5.90 (ddd, 1H, *J* = 5.6 Hz, 10.6 Hz, 17.1 Hz, CH allyl), 6.78 - 6.81 (m, 4H, H<sub>arom</sub>), 7.17 - 7.40 (m, 15H, H<sub>arom</sub>), 7.60 - 7.65 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 19.2 (C<sub>q</sub> *t*-Bu), 26.8 (3 x CH<sub>3</sub> TBDPS), 55.2 (2 x OMe), 62.5 (C-2), 63.2 (C-6), 63.9 (CH<sub>2</sub> glycerol), 69.8 (C-5), 70.0 (CH<sub>2</sub> glycerol), 71.0 (C-3), 72.4 (CH<sub>2</sub> allyl), 72.6 (C-4), 77.3 (CH glycerol), 86.4 (C<sub>q</sub> DMTr), 97.2 (C-1), 113.2 (CH<sub>arom</sub>), 117.0 (CH<sub>2</sub> allyl), 126.9 - 129.9 (CH<sub>arom</sub>), 133.1, 133.2 (2 x C<sub>q</sub> TBDPS), 134.6 (CH allyl), 135.6 (CH<sub>arom</sub>), 135.6, 144.4, 158.5 (5 x C<sub>q</sub> DMTr); HRMS: C<sub>49</sub>H<sub>57</sub>N<sub>3</sub>O<sub>9</sub>Si+Na<sup>+</sup> requires 882.3756, found 882.3764. To a solution of the intermediate diol (840 mg, 0.977 mmol) in THF (10 ml) was added TBAF (1.00 M in THF, 1.50 ml). This mixture was stirred overnight and concentrated *in vacuo*. Purification with column chromatography (EtOAc/PE, containing ~0.5% Et<sub>3</sub>N) furnished triol **33** (508 mg, 0.817 mmol, 84 %) as a colourless oil. [α]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +38.0; IR (neat): 1038, 1074, 1105, 1497, 2104, 2857, 2901, 2928, 3067; <sup>1</sup>H NMR (400 MHz):  $\delta$  = 3.12 (dd, 1H, *J* = 3.6 Hz, 10.4 Hz, H-2), 3.31 (dd, 1H, *J* = 5.1 Hz, 10.1 Hz, H-6), 3.38 (dd, 1H, *J* = 3.5 Hz, 10.1 Hz, H-6'), 3.49 - 3.78 (m, 11H,

H-4, 2 x OMe, 2 x CH<sub>2</sub> glycerol), 3.90 - 4.01 (m, 5H, H-3, H-5, CH<sub>2</sub> allyl, CH glycerol), 5.16 - 5.20 (m, 2H, H-1, CHH allyl), 5.27 (dd, 1H, *J* = 1.6 Hz, 17.2 Hz, CHH allyl), 5.89 (ddd, 1H, *J* = 5.6 Hz, 11.0 Hz, 17.3 Hz, CH allyl), 6.82 (d, 4H, *J* = 8.9 Hz, H<sub>arom</sub>), 7.15 - 7.33 (m, 7H, H<sub>arom</sub>), 7.43 (d, 2H, *J* = 7.4 Hz, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 55.2 (2 x OMe), 62.7 (C-2), 63.4 (C-6), 63.6 (CH<sub>2</sub> glycerol), 69.8 (CH<sub>2</sub> glycerol), 70.6 (C-5), 71.1 (C-4), 72.3 (C-3), 72.4 (CH<sub>2</sub> allyl), 78.1 (CH glycerol), 86.5 (C<sub>q</sub> DMTr), 97.5 (C-1), 113.1 (CH<sub>arom</sub>), 117.3 (CH<sub>2</sub> allyl), 126.9 - 130.0 (CH<sub>arom</sub>), 134.2 (CH allyl), 135.7, 135.8, 144.5, 158.5, 158.5 (5 x C<sub>q</sub> DMTr); HRMS: C<sub>33</sub>H<sub>39</sub>N<sub>3</sub>O<sub>9</sub>+Na<sup>+</sup> requires 644.2580, found 644.2579.



**3-O-Allyl-2-O-(2-azido-3,4-di-O-benzyl-2-deoxy-6-O-[4,4'-dimethoxytrityl]-α-D-glucopyranosyl)-1-O-benzyl-*sn*-glycerol (34)**

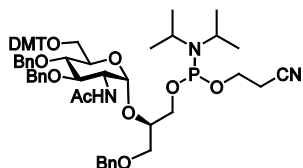
To a cooled (0° C) solution of triol **33** (4.08 g, 6.56 mmol) in DMF (35 ml) were added sodium hydride (60% in mineral oil, 944 mg, 23.7 mmol) and benzyl bromide (2.83 ml, 23.7 mmol). This reaction mixture was allowed to stir overnight at rt before MeOH was added (10 ml). After stirring for 30 min the reaction mixture was diluted with Et<sub>2</sub>O (250 ml) and washed with water (2 x 50 ml) and brine (100 ml). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*, after which the residue was purified by column chromatography (EtOAc/PE, containing ~0.5% Et<sub>3</sub>N) yielding fully protected construct **34** (5.45 g, 6.11 mmol, 93%) as a colourless oil. [α]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +48.6; IR (neat): 1034, 1250, 1508, 1607, 2106, 2851, 2922; <sup>1</sup>H NMR (400 MHz): δ = 3.10 (dd, 1H, *J* = 3.4 Hz, 10.3 Hz, H-6), 3.40 (dd, 1H, *J* = 1.4 Hz, 10.3 Hz, H-6'), 3.45 (dd, 1H, *J* = 3.6 Hz, 10.3 Hz, H-2), 3.56 - 3.68 (m, 4H, 2 x CH<sub>2</sub> glycerol), 3.73 - 3.75 (m, 6H, 2 x OMe), 3.83 (at, 1H, *J* = 9.5 Hz, H-4), 3.98 (at, 1H, *J* = 10.0 Hz, H-3), 4.02 - 4.09 (m, 3H, H-5, CH<sub>2</sub> allyl), 4.10 - 4.16 (m, 1H, CH glycerol), 4.29 (d, 1H, *J* = 10.4 Hz, CHH Bn), 4.43 (s, 2H, CH<sub>2</sub> Bn), 4.65 (d, 1H, *J* = 10.4 Hz, CHH Bn), 4.86 (s, 2H, CH<sub>2</sub> Bn), 5.21 (dd, 1H, *J* = 1.3 Hz, 10.4 Hz, CHH allyl), 5.29 - 5.34 (m, 2H, H-1, CHH allyl), 5.94 (ddd, 1H, *J* = 5.6 Hz, 10.7 Hz, 17.4 Hz, CH allyl), 6.74 - 6.78 (m, 4H, H<sub>arom</sub>), 6.82 - 6.86 (m, 2H, H<sub>arom</sub>), 7.14 - 7.47 (m, 22H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 55.1 (2 x OMe), 61.5 (C-6), 63.6 (C-2), 70.3, 70.4 (2 x CH<sub>2</sub> glycerol), 70.8 (C-5), 72.4 (CH<sub>2</sub> allyl), 73.1, 74.8 (2 x CH<sub>2</sub> Bn), 75.3 (CH glycerol), 75.6 (CH<sub>2</sub> Bn), 78.6 (C-4), 80.1 (C-3), 85.6 (C<sub>q</sub> DMTr), 97.4 (C-1), 113.0 (CH<sub>arom</sub>), 117.1 (CH<sub>2</sub> allyl), 126.6 - 129.1 (CH<sub>arom</sub>), 130.1, 130.2 (CH<sub>arom</sub>), 134.6 (CH allyl), 135.7, 136.2, 137.8, 138.0, 144.9, (3 x C<sub>q</sub> Bn, 5 x C<sub>q</sub> DMTr), 158.3, 158.4 (2 x C<sub>q</sub> DMTr); HRMS: C<sub>54</sub>H<sub>57</sub>N<sub>3</sub>O<sub>9</sub>+Na<sup>+</sup> requires 914.3987, found 914.3995.



**3-O-Allyl-2-O-(2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-[4,4'-dimethoxytrityl]-α-D-glucopyranosyl)-1-O-benzyl-*sn*-glycerol (35)**

To a solution of compound **34** (5.45 g, 6.11 mmol) in a mixture of 1,4-dioxane/water (36 ml, 5:1) was added PMe<sub>3</sub> (1.0 M in toluene, 12.2 ml, 12.2 mmol). After stirring for 4 h the mixture was concentrated *in vacuo* and coevaporated with toluene (3 x 50 ml). The crude intermediate was taken up in pyridine (30 ml) and Ac<sub>2</sub>O (1.13 ml, 12.0 mmol) was added. After stirring overnight, the mixture was diluted with EtOAc (300 ml) and washed with water (50 ml), saturated aqueous NaHCO<sub>3</sub> solution (50 ml) and brine (50 ml). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*, after which purification by column chromatography (EtOAc/PE, containing ~0.5% Et<sub>3</sub>N) yielded acetamide **35** (4.77 g, 5.25 mmol, 86%) as a slightly yellow oil. [α]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +30.0; IR (neat): 1090, 1250, 1454, 1508, 2853, 2924, 3063; <sup>1</sup>H NMR (400 MHz): δ = 1.87 (s, 3H, HNAC), 3.17 - 3.23 (m, 1H, H-6), 3.41 - 3.65 (m, 12H, H-3, H-6', 2 x CH<sub>2</sub> glycerol, 2 x OMe), 3.71 - 3.78 (m, 1H, H-4), 3.86 - 4.09 (m, 4H, H-5, CH glycerol, CH<sub>2</sub> allyl), 4.30 - 4.46 (m, 4H, H-2, CH<sub>2</sub>Bn, CHH Bn), 4.65 - 4.72 (m, 2H, 2 x CHH Bn), 4.85 (d, 1H, *J* = 11.5 Hz, CHH Bn), 5.08 - 5.25 (m, 3H, H-1, CH<sub>2</sub> allyl), 5.82 (ddd, 1H, *J* = 5.4 Hz, 10.5 Hz, 16.1 Hz, CH allyl), 6.09 (d, 1H, *J* = 9.0 Hz, HNAC), 6.73 - 6.77 (m, 4H, H<sub>arom</sub>), 6.86 - 6.89 (m, 2H, H<sub>arom</sub>), 7.09 - 7.40 (m, 20H, H<sub>arom</sub>),

7.52 (d, 2H,  $J = 7.5$  Hz,  $H_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 22.8$  (HNAC), 52.6 (C-2), 54.4 (2 x OMe), 61.3 (C-6), 69.6, 69.7 (2 x  $\text{CH}_2$  glycerol), 70.8 (C-5), 71.6 ( $\text{CH}_2$  allyl), 72.5, 74.2, 74.5 (3 x  $\text{CH}_2$  Bn), 76.0 (CH glycerol), 78.0 (C-4), 80.3 (C-3), 85.1 ( $\text{C}_q$  DMTr), 97.8 (C-1), 112.5 ( $\text{CH}_{\text{arom}}$ ), 116.6 ( $\text{CH}_2$  allyl), 126.0 - 127.8 ( $\text{CH}_{\text{arom}}$ ), 129.6, 129.7 ( $\text{CH}_{\text{arom}}$ ), 133.8 (CH allyl), 135.2, 135.6, 137.3, 137.4, 138.1, 144.6, 157.8 (3 x  $\text{C}_q$  Bn, 5 x  $\text{C}_q$  DMTr), 169.3 (C=O HNAC); HRMS:  $\text{C}_{56}\text{H}_{61}\text{NO}_{10} + \text{Na}^+$  requires 930.4188, found 930.4195.

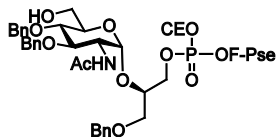


**3-([N,N-diisopropylamino]-2-cyanoethylphosphite)-2-O-((2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-[4,4'-dimethoxytrityl]-α-D-glucopyranosyl)-1-O-benzyl-sn-glycerol) (36)**

A solution of compound 35 (6.36 g, 7.00 mmol) in THF (100 ml) was stirred under argon for 30 min after which  $\text{Ir}(\text{COD})(\text{PPhMe})_2\text{PF}_6$  (177 mg, 0.209 mmol) was added. The solution was purged with  $\text{H}_2$  (g) (20s) before it was stirred under argon for 1 h. After the addition of, respectively, saturated aqueous  $\text{NaHCO}_3$  solution (25 ml) and  $\text{I}_2$  (4.80 g, 18.9 mmol) the mixture was stirred for 30 min before it was diluted with  $\text{EtOAc}$  (400 ml) and washed with saturated  $\text{Na}_2\text{S}_2\text{O}_3$  solution (100 ml) and brine (200 ml). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo*, after which purification by column chromatography ( $\text{EtOAc}/\text{PE}$ , containing ~0.5%  $\text{Et}_3\text{N}$ ) yielded the intermediate alcohol (5.63 g, 6.49 mmol, 93%) as a yellow foam. Analytical data **1-O-benzyl-2-O-((2-acetamido-3,4-di-O-benzyl-2-deoxy-6-O-[4,4'-dimethoxytrityl]-α-D-glucopyranosyl)-sn-glycerol)**:  $[\alpha]_{\text{D}}^{20}$

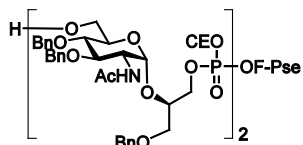
( $\text{CHCl}_3$ ): +49.8; IR (neat): 1028, 1091, 1246, 1660, 2945;  $^1\text{H}$  NMR (400 MHz):  $\delta = 1.86$  (s, 3H, HNAC), 2.70 (bs, 1H, OH), 3.19 (dd, 1H,  $J = 4.0, 10.2$  Hz, H-6), 3.42 (dd, 1H,  $J = 1.3, 10.0$  Hz, H-6'), 3.59 (dd, 1H,  $J = 5.3$  Hz, 10.0 Hz,  $\text{CHH}$  glycerol), 3.64 - 3.79 (m, 10H, H-3,  $\text{CH}_2$  glycerol,  $\text{CHH}$  glycerol, 2 x OMe), 3.79 - 3.90 (m, 2H, H-4, CH glycerol), 3.95 (dd, 1H,  $J = 2.2$  Hz, 9.9 Hz, H-5), 4.26 - 4.33 (m, 2H, H-2,  $\text{CHH}$  Bn), 4.43 (s, 2H,  $\text{CH}_2$  Bn), 4.61 - 4.67 (m, 2H,  $\text{CH}_2$  Bn), 4.84 (d, 1H,  $J = 11.6$  Hz,  $\text{CHH}$  Bn), 5.09 (d, 1H,  $J = 3.6$  Hz, H-1), 6.16 (d, 1H,  $J = 8.8$  Hz, HNAC), 6.76 - 6.81 (m, 4H,  $H_{\text{arom}}$ ), 6.84 - 6.88 (m, 2H,  $H_{\text{arom}}$ ), 7.15 - 7.37 (m, 20H,  $H_{\text{arom}}$ ), 7.46 - 7.49 (m, 2H,  $H_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 23.2$  (HNAC), 53.1 (C-2), 55.1 (2 x OMe), 61.9 (C-6), 62.6, 70.5 (2 x  $\text{CH}_2$  glycerol), 71.4 (C-5), 73.4, 74.9, 75.0 (3 x  $\text{CH}_2$  Bn), 77.2 (CH glycerol), 78.4 (C-4), 80.3 (C-3), 85.7 ( $\text{C}_q$  DMTr), 97.5 (C-1), 113.0 ( $\text{CH}_{\text{arom}}$ ), 126.6 - 130.2 ( $\text{CH}_{\text{arom}}$ ), 135.8, 136.1, 137.5, 137.7, 138.4, 144.9, 158.4 (3 x  $\text{C}_q$  Bn, 5 x  $\text{C}_q$  DMTr), 170.5 (C=O); HRMS:  $\text{C}_{53}\text{H}_{57}\text{NO}_{10} + \text{Na}^+$  requires 890.3875, found 890.3880. To a solution of the intermediate alcohol (694 mg, 0.800 mmol) and  $\text{Et}_3\text{N}$  (166  $\mu\text{l}$ , 1.20 mmol) in DCM (8.0 ml) was added (*N,N*-diisopropylamino)-2-cyanoethyl-chlorophosphoramidite (214  $\mu\text{l}$ , 0.959 mmol). This mixture was allowed to stir for 2 h, after which  $\text{H}_2\text{O}$  (2.0 ml) was added. The mixture was diluted with DCM (20 ml) and washed was washed with brine (15 ml). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo*, after which the residue was purified by column chromatography ( $\text{EtOAc}/\text{PE}$ , containing ~0.5%  $\text{Et}_3\text{N}$ ) yielding phosphoramidite **36** (786 mg, 0.736 mmol, 92%, mixture of diastereoisomers) as a white foam. IR (neat): 1248, 1454, 1508, 1653, 2837, 2870, 2928, 2965, 3021, 3055, 3221;  $^{31}\text{P}$  NMR (162 MHz):  $\delta = 147.3, 147.8$  (diastereoisomers);  $^1\text{H}$  NMR (400 MHz):  $\delta = 1.21 - 1.24$  (m, 12 H, 4 x  $\text{CH}_3$  isopropylamino), 1.95 - 1.97 (m, 3H,  $J = 1.6$  Hz, HNAC), 2.64 - 2.68 (m, 2H,  $\text{CH}_2$  cyanoethyl), 3.07 (dd, 1H,  $J = 4.7, 10.1$  Hz, H-6), 3.38 (ad, 1H,  $J = 9.9$  Hz, H-6'), 3.61 - 3.90 (m, 16H, H-3, H-4, 2 x CH isopropylamino, 2 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$  cyanoethyl, 2 x OMe), 4.09 - 4.15 (m, 2H, H-5, CH glycerol), 4.17 - 4.26 (m, 1H, H-2), 4.30 (d, 1H,  $J = 10.7$  Hz,  $\text{CHH}$  Bn), 4.47 - 4.54 (m, 2H,  $\text{CH}_2$  Bn), 4.66 - 4.73 (m, 2H,  $\text{CH}_2$  Bn), 4.79 - 4.84 (m, 1H,  $\text{CHH}$  Bn), 5.10 (d, 0.5H,  $J = 3.6$  Hz, H-1 diastereoisomer 1), 5.12 (d, 0.5H,  $J = 3.6$  Hz, H-1 diastereoisomer 2), 6.47 (d, 0.5H,  $J = 9.5$  Hz, HNAC diastereoisomer 1), 6.53 (d, 0.5H,  $J = 9.6$  Hz, HNAC diastereoisomer 2), 6.82 - 6.87 (m, 4H,  $H_{\text{arom}}$ ), 6.92 - 6.96 (m, 2H,  $H_{\text{arom}}$ ), 7.20 - 7.38 (m, 20H,  $H_{\text{arom}}$ ), 7.52 (d, 2H,  $J = 7.5$  Hz,  $H_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta = 21.0, 21.1$  ( $\text{CH}_2$  cyanoethyl), 23.5 (HNAC), 24.9 - 25.1 (4 x  $\text{CH}_3$

isopropylamino), 43.8, 43.9 (2 x CH isopropylamino), 53.9, 53.9 (C-2), 55.8 (2 x OMe), 59.3 - 59.6 (CH<sub>2</sub> cyanoethyl), 63.3 (C-6), 63.8, 63.9 (CH<sub>2</sub> glycerol) 71.1 (CH<sub>2</sub> glycerol), 71.9 (C-5), 73.6, 75.2, 75.8 (3 x CH<sub>2</sub> Bn), 76.7 - 76.9 (CH glycerol), 79.6 (C-4), 81.7, 81.8 (C-3), 86.5 (C<sub>q</sub> DMTr), 98.0, 98.2 (C-1), 114.0 (CH<sub>arom</sub>), 118.3 (C<sub>q</sub> cyanoethyl), 127.6 - 129.2 (CH<sub>arom</sub>), 131.0, 131.1 (CH<sub>arom</sub>), 136.8, 137.1, 139.2, 139.5, 139.9, 146.3, 159.5 (3 x C<sub>q</sub> Bn, 5 x C<sub>q</sub> DMTr), 170.5 (C=O NHAc); HRMS: C<sub>62</sub>H<sub>74</sub>N<sub>3</sub>O<sub>11</sub>P+H<sup>+</sup> requires 1068.5134, found 1068.5146.



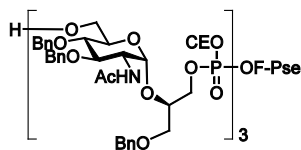
### GlcNAc-glycerol phosphate monomer (37)

Fluorous alcohol **5** (254 mg, 445 μmol) was coupled to phosphoramidite **36** (0.2 M in MeCN, 3.34 ml, 668 μmol, 1.5 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Monomer **37** (501 mg, 400 μmol, 90 %) was obtained as a white foam. <sup>31</sup>P NMR (161.7 MHz): δ = -1.8, -1.6 (1P); <sup>1</sup>H NMR (400 MHz): δ = 1.95 (s, 3H, NHAc), 2.00 - 2.39 (m, 5H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub>OH), 2.58 - 2.76 (m, 2H, cyanoethyl), 3.00 (t, 1H, *J* = 7.4 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CHHSO<sub>2</sub>-), 3.14 (t, 1H, *J* = 7.4 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CHHSO<sub>2</sub>-), 3.25 - 3.40 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.52 - 3.79 (m, 7H, H-3, H-4, H-5, H-6, H-6', CH<sub>2</sub> glycerol), 3.88 - 3.92 (m, 1H, CH glycerol), 4.13 - 4.37 (m, 5H, H-2, CH<sub>2</sub> cyanoethyl, CH<sub>2</sub> glycerol), 4.43 - 4.52 (m, 4H, CH<sub>2</sub> Bn, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 4.63 (d, 1H, *J* = 10.8 Hz, CHH Bn), 4.71 (d, 1H, *J* = 11.4 Hz, CHH Bn), 4.79 - 4.86 (m, 2H, 2 x CHH Bn), 4.91 (m, 1H, H-1), 6.87 (d, 0.5 H, *J* = 9.6 Hz, NHAc), 6.91 (d, 0.5 H, *J* = 9.5 Hz, NHAc), 7.24 - 7.37 (m, 15H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 13.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 19.4 - 19.6 (CH<sub>2</sub> cyanoethyl), 23.0 (NHAc), 29.1 - 29.6 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 52.7 (C-2), 52.9 - 53.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 61.1 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 61.6 (C-6), 62.4 - 62.6 (CH<sub>2</sub> cyanoethyl), 68.3 - 68.7 (2 x CH<sub>2</sub> glycerol), 72.1 (C-5), 73.5 (CH<sub>2</sub> Bn), 74.8 - 75.1 (2 x CH<sub>2</sub> Bn), 76.8 (CH glycerol), 77.7, 77.8 (C-4), 80.3 (C-3), 99.6 (C-1), 116.5 (C<sub>q</sub> cyanoethyl), 127.6 - 128.5 (CH<sub>arom</sub>), 137.4, 137.9, 138.4 (3 x C<sub>q</sub> Bn), 170.7 (C<sub>q</sub> acetyl); HRMS: C<sub>48</sub>H<sub>52</sub>F<sub>17</sub>N<sub>2</sub>O<sub>13</sub>PS+H<sup>+</sup> requires 1252.2763, found 1252.2764.

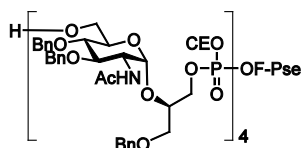


### GlcNAc-glycerol phosphate dimer (38)

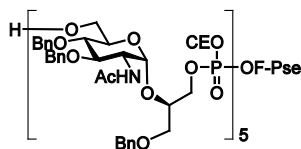
Monomer **37** (250 mg, 200 μmol) was coupled to phosphoramidite building-block **36** (0.2M in MeCN, 1.50 ml, 300 μmol, 1.5 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Dimer **38** (321 mg, 166 μmol, 83 %) was obtained as a white foam. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.9 (0.5P), -1.7, -1.7 (0.5P), -1.1, -1.1 (0.5P), -0.9, -0.9 (0.5P); <sup>1</sup>H NMR (400 MHz): δ = 1.94 - 1.99 (m, 6H, 2 x NHAc), 2.03 - 2.37 (m, 5H, CH<sub>2</sub>OH, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 2.54 - 2.76 (m, 4H, 2 x CH<sub>2</sub> cyanoethyl), 2.93 (t, 0.5H, *J* = 7.5 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CHHSO<sub>2</sub>-), 3.02 (t, 0.5H, *J* = 7.4 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CHHSO<sub>2</sub>-), 3.13 (t, 1H, *J* = 7.1 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CHHSO<sub>2</sub>-), 3.23 - 3.41 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.46 - 3.85 (m, 12H, 2 x H-3, 2 x H-4, 2 x H-5, H-6, H-6', 2 x CH<sub>2</sub> glycerol), 3.89 - 3.96 (m, 2H, 2 x CH glycerol), 4.00 - 4.33 (m, 12H, 2 x H-2, H-6, H-6', 2 x CH<sub>2</sub> glycerol, 2 x CH<sub>2</sub> cyanoethyl), 4.39 - 4.52 (m, 6H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, 2 x CH<sub>2</sub> Bn), 4.58 - 4.93 (m, 10H, 2 x H-1, 4 x CH<sub>2</sub> Bn), 6.81 - 7.15 (m, 2H, 2 x NH), 7.22 - 7.37 (m, 30H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 13.2 - 13.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 19.2 - 19.5 (2 x CH<sub>2</sub> cyanoethyl), 22.9 (2 x NHAc), 28.9 - 29.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 52.5, 52.6 (2 x C-2), 52.9 - 53.2 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 61.0 - 61.1 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 61.4 - 62.5 (C-6, 2 x CH<sub>2</sub> cyanoethyl), 66.3 - 66.6 (C-6), 67.9 - 68.6 (4 x CH<sub>2</sub> glycerol), 70.2 (CH glycerol), 72.1, 72.5 (2 x C-5), 73.3 (2 x CH<sub>2</sub> Bn), 74.9 - 75.1 (4 x CH<sub>2</sub> Bn), 76.3 - 76.6 (CH glycerol), 77.6, 78.3 (2 x C-4), 80.1 - 80.5 (2 x C-3), 99.0 - 99.7 (2 x C-1), 116.5 (2 x C<sub>q</sub> cyanoethyl), 127.4 - 128.4 (CH<sub>arom</sub>), 137.3 - 138.3 (6 x C<sub>q</sub> Bn), 170.7 - 170.8 (2 x C<sub>q</sub> acetyl); HRMS: C<sub>83</sub>H<sub>93</sub>F<sub>17</sub>N<sub>4</sub>O<sub>23</sub>P<sub>2</sub>S+H<sup>+</sup>, requires 1931.5228, found 1931.5237.

**GlcNAc-glycerol phosphate trimer (39)**

Dimer **38** (312 mg, 162  $\mu\text{mol}$ ) was coupled to phosphoramidite building-block **36** (0.2 M in MeCN, 1.22 ml, 243  $\mu\text{mol}$ , 1.5 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Trimer **39** (336 mg, 129  $\mu\text{mol}$ , 80 %) was obtained as a white foam.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta$  = -1.9, -1.9 (0.5P), -1.7, -1.7 (0.5P), -1.3, -1.2, -1.2, -1.1 (1P), -0.9, -0.9 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.93 - 2.02 (m, 9H, 3 x NHAc), 2.06 - 2.33 (m, 4H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -), 2.47 - 2.73 (m, 7H, 3 x  $\text{CH}_2$  cyanoethyl,  $\text{CH}_2\text{OH}$ ), 2.94 - 3.05 (m, 1H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CHHSO}_2$ -), 3.12 (t, 1H, 7.0 Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CHHSO}_2$ -), 3.21 - 3.38 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2$ -), 3.45 - 3.86 (m, 17H, 3 x H-3, 3 x H-4, 3 x H-5, H-6, H-6', 3 x  $\text{CH}_2$  glycerol), 3.88 - 3.99 (m, 3H, 3 x CH glycerol), 4.00 - 4.35 (m, 19H, 3 x H-2, 2 x H-6, 2 x H-6', 3 x  $\text{CH}_2$  glycerol, 3 x  $\text{CH}_2$  cyanoethyl), 4.38 - 4.51 (m, 8H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2$ -, 3 x  $\text{CH}_2$  Bn), 4.55 - 4.93 (m, 15H, 3 x H-1, 6 x  $\text{CH}_2$  Bn), 6.95 - 7.60 (m, 48H, 3 x NHAc,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 13.2 - 13.4 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -), 19.2 - 19.6 (3 x  $\text{CH}_2$  cyanoethyl), 22.9 (NHAc), 29.2 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -), 52.5 - 52.6 (3 x C-2), 53.0 - 53.2 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -,  $-\text{OCH}_2\text{CH}_2\text{SO}_2$ -), 61.0 - 61.1 ( $-\text{OCH}_2\text{CH}_2\text{SO}_2$ -), 61.4 - 62.5 (C-6, 3 x  $\text{CH}_2$  cyanoethyl), 66.3 - 66.9 (2 x C-6), 67.9 - 68.6 (6 x  $\text{CH}_2$  glycerol), 70.1 - 70.2 (2 x CH glycerol), 72.0 - 72.5 (3 x C-5), 73.2 - 73.4 (3 x  $\text{CH}_2$  Bn), 74.8 - 75.2 (6 x  $\text{CH}_2$  Bn), 76.9 (CH glycerol), 77.1, 77.6, 78.5 (3 x C-4), 80.3 - 80.6 (3 x C-3), 99.1 - 99.8 (3 x C-1), 116.4 - 116.5 (3 x  $\text{C}_q$  cyanoethyl), 127.4 - 128.5 ( $\text{CH}_{\text{arom}}$ ), 137.3 - 138.4 (9 x  $\text{C}_q$  Bn), 170.7 - 170.8 (3 x  $\text{C}_q$  acetyl); HRMS:  $[\text{C}_{118}\text{H}_{134}\text{F}_{17}\text{N}_6\text{O}_{33}\text{P}_3\text{S}+2\text{NH}_4]^{2+}$  requires 1323.9182, found 1323.9186.

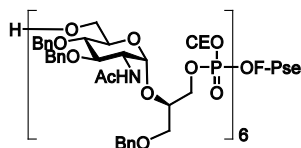
**GlcNAc-glycerol phosphate tetramer (40)**

Trimer **39** (252 mg, 96.4  $\mu\text{mol}$ ) was coupled to phosphoramidite building-block **36** (0.2 M in MeCN, 0.72 ml, 144  $\mu\text{mol}$ , 1.5 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Tetramer **40** (225 mg, 68.2  $\mu\text{mol}$ , 71 %) was obtained as a white foam.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta$  = -1.9, -1.9 (0.5P), -1.7, -1.7 (0.5P), -1.3 - -1.1 (1.5P), -0.9 - -0.9 (1.5P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.93 - 2.01 (m, 12H, 4 x NHAc), 2.08 - 2.37 (m, 5H,  $\text{CH}_2\text{OH}$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -), 2.52 - 2.74 (m, 8H, 4 x  $\text{CH}_2$  cyanoethyl), 2.94 - 3.15 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -), 3.23 - 3.38 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2$ -), 3.45 - 3.89 (m, 22H, 4 x H-3, 4 x H-4, 4 x H-5, H-6, H-6', 4 x  $\text{CH}_2$  glycerol), 3.90 - 3.98 (m, 4H, 4 x CH glycerol), 3.99 - 4.35 (m, 26H, 4 x H-2, 3 x H-6, 3 x H-6', 4 x  $\text{CH}_2$  glycerol, 4 x  $\text{CH}_2$  cyanoethyl), 4.37 - 4.51 (m, 10H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2$ -, 4 x  $\text{CH}_2$  Bn), 4.55 - 4.93 (m, 20H, 4 x H-1, 8 x  $\text{CH}_2$  Bn), 6.94 - 7.62 (m, 64H, 4 x NHAc,  $\text{H}_{\text{arom}}$ ); HRMS:  $[\text{C}_{153}\text{H}_{175}\text{F}_{17}\text{N}_8\text{O}_{43}\text{P}_4\text{S}+2\text{H}]^{2+}$  requires 1647.0166, found 1647.0176.

**GlcNAc-glycerol phosphate pentamer (41)**

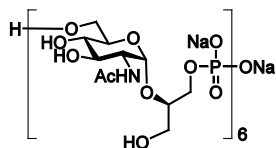
Tetramer **40** (218 mg, 66.1  $\mu\text{mol}$ ) was coupled to phosphoramidite building-block **36** (0.2 M in MeCN, 0.59 ml, 119  $\mu\text{mol}$ , 1.8 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Pentamer **41** (199 mg, 50.0  $\mu\text{mol}$ , 76 %) was obtained as a white foam.  $^{31}\text{P}$  NMR (161.7 MHz):  $\delta$  = -1.9, -1.9 (0.5P), -1.7, -1.7 (0.5P), -1.2 - -1.1 (2P), -1.0 - -0.9 (2P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.94 - 2.02 (m, 15H, 5 x NHAc), 2.08 - 2.36 (m, 5H,  $\text{CH}_2\text{OH}$ ,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -), 2.49 - 2.74 (m, 10H, 5 x  $\text{CH}_2$  cyanoethyl), 2.94 - 3.15 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{SO}_2$ -), 3.23 - 3.38 (m, 2H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2$ -), 3.44 - 3.87 (m, 27H, 5 x H-3, 5 x H-4, 5 x H-5, H-6, H-6', 5 x  $\text{CH}_2$  glycerol), 3.88 - 3.98 (m, 5H, 5 x CH glycerol), 3.99 - 4.36 (m, 33H, 5 x H-2, 4 x H-6, 4 x H-6', 5 x  $\text{CH}_2$  glycerol, 5 x  $\text{CH}_2$  cyanoethyl), 4.37 - 4.52 (m, 12H,  $-\text{OCH}_2\text{CH}_2\text{SO}_2$ -, 5 x

CH<sub>2</sub> Bn), 4.55 - 4.93 (m, 25H, 5 x H-1, 10 x CH<sub>2</sub> Bn), 6.95 - 7.65 (m, 80H, 5 x NHAc, H<sub>arom</sub>); HRMS: [C<sub>188</sub>H<sub>216</sub>F<sub>17</sub>N<sub>10</sub>O<sub>53</sub>P<sub>5</sub>S+2H]<sup>2+</sup> requires 1987.6432, found 1987.6437.



#### GlcNAc-glycerol phosphate hexamer (42)

Pentamer **41** (186 mg, 46.9  $\mu$ mol) was coupled to phosphoramidite building-block **36** (0.2 M in MeCN, 0.47 ml, 93.8  $\mu$ mol, 2.0 eq), oxidized, detritylated and purified (F-SPE) using the general procedure as described above. Hexamer **42** (183 mg, 39.3  $\mu$ mol, 84 %) was obtained as a white foam. <sup>31</sup>P NMR (161.7 MHz):  $\delta$  = -1.9, -1.9, (0.5P), -1.7, -1.7 (0.5P), -1.3 - -1.1 (2.5P), -1.0 - -0.9 (2.5P); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 1.93 - 2.02 (m, 18H, 6 x NHAc), 2.09 - 2.37 (m, 5H, CH<sub>2</sub>OH, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 2.49 - 2.74 (m, 12H, 6 x CH<sub>2</sub> cyanoethyl), 2.94 - 3.16 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>), 3.22 - 3.38 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.44 - 3.87 (m, 32H, 6 x H-3, 6 x H-4, 6 x H-5, H-6, 6 x CH<sub>2</sub> glycerol), 3.89 - 3.98 (m, 6H, 6 x CH glycerol), 3.98 - 4.35 (m, 40H, 6 x H-2, 5 x H-6, 5 x H-6', 6 x CH<sub>2</sub> glycerol, 6 x CH<sub>2</sub> cyanoethyl), 4.37 - 4.52 (m, 14H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, 6 x CH<sub>2</sub> Bn), 4.54 - 4.93 (m, 30H, 6 x H-1, 12 x CH<sub>2</sub> Bn), 6.90 - 7.61 (m, 96H, 6 x NHAc, H<sub>arom</sub>); HRMS: [C<sub>223</sub>H<sub>257</sub>F<sub>17</sub>N<sub>12</sub>O<sub>63</sub>P<sub>6</sub>S+2H]<sup>2+</sup> requires 2327.7681, found 2327.7673.



#### GlcNAc-glycerol phosphate hexamer (43)

Protected hexamer **42** (85.7 mg, 18.4  $\mu$ mol) was treated with aqueous ammonia as described above affording the intermediate hexamer (71.6 mg, 18.3  $\mu$ mol, 100%). Analytical data intermediate: <sup>31</sup>P NMR (161.7 MHz):  $\delta$  = 0.5 - 0.9 (6P); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 1.72 - 1.98 (m, 18H, 6 x NHAc), 2.89, 3.35 (2 x t, 2 x 12H,  $J$  = 6.8 Hz, 6 x NH<sub>4</sub>), 3.35 - 4.65 (m, 102H, 6 x H-2, 6 x H-3, 6 x H-4, 6 x H-5, 6 x H-6, 6 x H-6', 6 x CH glycerol, 12 x CH<sub>2</sub> glycerol, 18 x CH<sub>2</sub> Bn), 4.86 - 5.02 (m, 6H, 6 x H-1), 6.67 - 7.22 (m, 90H, H<sub>arom</sub>); HRMS: [C<sub>192</sub>H<sub>230</sub>N<sub>6</sub>O<sub>61</sub>P<sub>6</sub>+H+NH<sub>4</sub>]<sup>2+</sup> requires 1901.1991, found 1901.2015. A portion of the semiprotected hexamer (69.6 mg, 17.8  $\mu$ mol) was deprotected with Pd (0)/H<sub>2</sub> using the standard procedure. Hexamer **43** (31.5 mg, 13.6  $\mu$ mol, 76%) was obtained as an amorphous white solid. <sup>31</sup>P NMR (161.7 MHz):  $\delta$  = 1.2 - 1.3 (5P), 3.0 (1P, phosphomonoester); <sup>1</sup>H NMR (600 MHz):  $\delta$  = 2.06 - 2.08 (m, 18H, 6 x NHAc), 3.48 (t, 1H,  $J$  = 9.4 Hz, H-3), 3.53 - 3.57 (m, 5H, 5 x H-3), 3.72 - 3.80 (m, 19H, 6 x H-4, H-6, 6 x CH<sub>2</sub> glycerol), 3.82 - 3.91 (m, 10H, H-5, H-6', 6 x CH glycerol, CH<sub>2</sub> glycerol), 3.92 - 4.03 (m, 21H, 6 x H-2, 6 x H-5, 5 x H-6, 5 x H-6'), 4.06 - 4.10 (m, 10H, 5 x CH<sub>2</sub> glycerol), 5.03 (d, 1H,  $J$  = 3.8 Hz, H-1), 5.04 (d, 4H,  $J$  = 3.6 Hz, H-1), 5.07 (d, 1H,  $J$  = 3.7 Hz, H-1); <sup>13</sup>C NMR (150 MHz):  $\delta$  = 23.0 - 23.1 (6 x NHAc), 54.5 (6 x C-2), 61.4 (C-6), 62.1 - 62.2 (6 x CH<sub>2</sub> glycerol), 63.9 (CH<sub>2</sub> glycerol), 65.1 - 65.4 (5 x C-6, 5 x CH<sub>2</sub> glycerol), 70.5 (5 x C-3), 70.9 (C-3), 71.9 - 72.1 (6 x C-4, 5 x C-5), 73.1 (C-5), 78.3 (d,  $J$  = 7.4 Hz, CH glycerol), 78.9 - 79.1 (4 x CH glycerol), 79.3 (d,  $J$  = 7.4 Hz, CH glycerol), 97.9 (C-1), 98.2 - 98.4 (5 x C-1), 175.4 (5 x C<sub>q</sub> acetyl), 175.6 (C<sub>q</sub> acetyl); HRMS: C<sub>66</sub>H<sub>122</sub>N<sub>6</sub>O<sub>61</sub>P<sub>6</sub>+NH<sub>4</sub><sup>+</sup> requires 2178.5393, found 2178.5405.

## References

- Hogendorf, W.F.J.; Van den Bos, L.J.; Overkleeft, H.S.; Codée, J.D.C.; van der Marel, G.A. *Bioorg. Med. Chem.* **2010**, *18*, 3668-3678.
- Hogendorf, W.F.J.; Meeuwenoord, N.; Overkleeft, H.S.; Filippov, D.V.; Laverde, D.; Kropec, A.; Huebner, J.; van der Marel, G.A.; Codée, J.D.C. *Chem. Commun.* **2011**, *47*, 8961-8963.
- Toy, P.H.; Janda, K.D. *Acc. Chem. Res.* **2000**, *33*, 546-554.
- Encinas, L.; Chiara, J.L. *Eur. J. Org. Chem.* **2009**, 2163-2173.
- He, X.; Chan, T.H. *Synthesis* **2006**, 1645-1651.
- Gladysz, J.A.; Curran, D.P.; Horváth, I.T., *Handbook of Fluorous Chemistry*; Eds: Wiley-VCH: Weinheim, **2004**.
- Curran, D.P. *Aldrichimica Acta* **2006**, *39*, 3-9.
- Zhang, W.; Curran, D.P. *Tetrahedron* **2006**, *62*, 11837-11865.
- Zhang, W.; Cai, C. *Chem. Commun.* **2008**, 5686-5694.
- Zhang, W. *Chem. Rev.* **2009**, *109*, 749-795.
- Zhang, W. *Green. Chem.* **2009**, *11*, 911-920.
- Manzoni, L.; Castelli, R. *Org. Lett.* **2006**, *8*, 955-957.
- Carrel, F.R.; Geyer, K.; Codée, J.D.C.; Seeberger, P.H. *Org. Lett.* **2007**, *9*, 2285-2288.
- Carrel, F.R.; Seeberger, P.H. *J. Org. Chem.* **2008**, *73*, 2058-2065.
- Cheng, Y.; Guo, A.-L.; Guo, D.-S. *Curr. Org. Chem.* **2010**, *14*, 977-999.
- Codée, J.D.C.; Ali, A.; Overkleeft, H.S.; Van der Marel, G.A. *C. R. Chim.* **2011**, *14*, 178-193.
- Tripathy, S.; Misra, K.; Sanghvi, Y.S. *Org. Prep. Proc.* **2005**, *37*, 257-263.
- Pearson, W.H.; Berry, D.A.; Stoy, P.; Jung, K.-Y.; Sercel, A.D. *J. Org. Chem.* **2005**, *70*, 7114-7122.
- Beller, C.; Bannwarth, W. *Helv. Chim. Acta* **2005**, *88*, 171-179.
- Hogendorf, W.F.J.; Lameijer, L.N.; Beenakker, T.J.M.; Overkleeft, H.S.; Filippov, D.V.; Codée, J.D.C.; Van der Marel, G.A. *Org. Lett.* **2012**, *14*, 848-851.
- Liu, L.; Pohl, N.L.B. *Org. Lett.* **2011**, *13*, 1824-1827.
- De Visser, P.C.; Van Helden, M.; Filippov, D.V.; Van der Marel, G.A.; Drijfhout, J.W.; Van Boom, J.H.; Noort, D.; Overkleeft, H.S. *Tetrahedron Lett.* **2003**, *44*, 9013-9016.
- Ali, A.; Van den Berg, R.J.B.H.N.; Overkleeft, H.S.; Filippov, D.V.; Van der Marel, G.A.; Codée, J.D.C. *Tetrahedron Lett.* **2009**, *50*, 2185-2188.
- Morath, S.; Geyer, A.; Hartung, T. *J. Exp. Med.* **2001**, *193*, 393-397.
- Tul'skaya, E.M.; Vylegzhanina, K.S.; Streshinskaya, G.M.; Shashkov, A.S.; Naumova, I.B. *Biochim. Biophys. Acta* **1991**, *1074*, 237-242.
- Kozlova, Y.I.; Streshinskaya, G.M.; Shashkov, A.S.; Evtushenko, L.I.; Naumova, I.B. *Biochem. (Mosc.)* **1999**, *64*, 671-677.
- Figueroa-Perez, I.; Stadelmaier, A.; Morath, S.; Hartung, T.; Schmidt, R.R. *Tetrahedron: Asymmetry* **2005**, *16*, 493-506.
- Shashkov, A.S.; Streshinskaya, G.M.; Evtushenko, L.I.; Naumova, I.B. *Carbohydr. Res.* **2001**, *336*, 237-242.





## Chapter 5

# Light Fluorous Synthesis of Glucosylated GTAs

### Introduction

The automated solid phase strategy described in **chapter three**, led to the generation of a small library of teichoic acid (TA) fragments. The antigenicity of these molecules was evaluated by means of an opsonophagocytic inhibition assay (OPIA). In this assay the inhibition of killing *Enterococcus faecalis* by rabbit antibodies raised against a biological preparation of native enterococcal LTA by the synthetic antigens was quantified. It was found that two glucosylated TA fragments showed the highest

inhibitory potency (**1** and **2**, figure 1) when tested in such an assay (see figures 2 and 3, **chapter three**).<sup>1</sup> A next step in the immunological evaluation of **1** and **2** comprises the coupling of these molecules to carrier proteins and test the potential of the resulting conjugates as a vaccine against *Enterococcus faecalis*.<sup>2-4</sup> In order to achieve this, sufficient quantities of **1** and **2** are required which cannot easily be generated using an automated solid phase synthesis approach.

In **chapter four**, a light fluorous<sup>5-10</sup> approach is described, which allowed the rapid production of multimilligram amounts of TAs. With this method, starting from

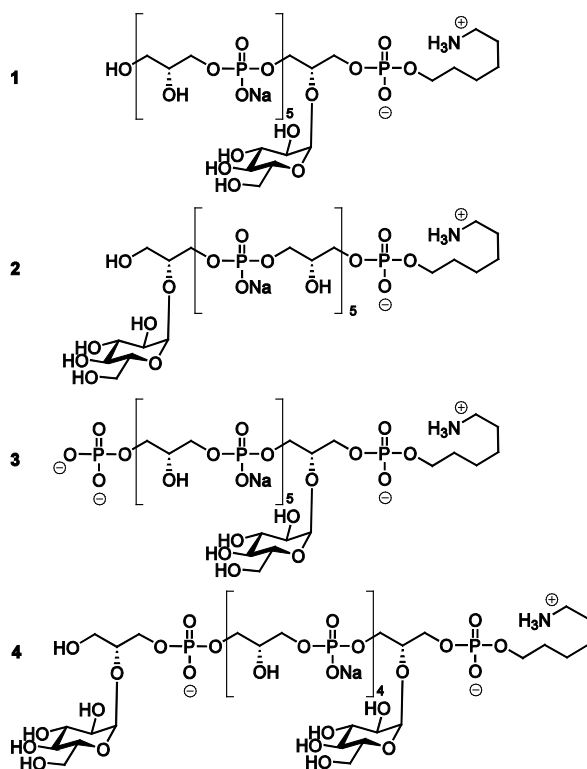


Figure 1. Glucosylated hexamers 1-4.

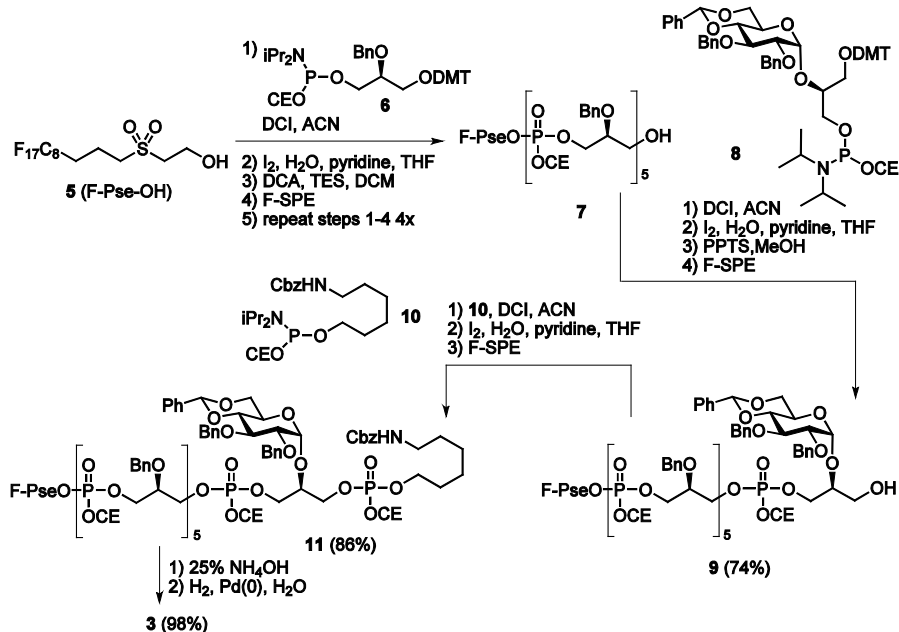
the perfluorooctylpropylsulfonyl ethanol linker (F-Pse), it was possible to perform a quick purification of the intermediates using fluorous solid phase extraction (F-SPE) after each elongation cycle. At the end of the synthesis, the fluorous linker is cleaved and the molecules undergo a final hydrogenolysis step, giving multimilligram quantities of the target TAs bearing a terminal phosphomonoester.<sup>11</sup>

This chapter first discusses the synthesis of TA fragment **3**, a phosphorylated version of hexamer **1**, which is attained using the earlier described light fluorous approach. To enable the light fluorous synthesis of TAs without terminal phosphate an alternative fluorous linker is required. The perfluorooctylpropyl succinyl linker proved suitable for this, as is illustrated by the synthesis of lead TA **2** and TA **4** in which a second glucosyl moiety is incorporated.<sup>12</sup> Finally, the antigenic properties of the prepared TAs (**2,3,4**) are probed in an OPIA, as described earlier, whereby the influence of a terminal phosphate and a second glucosyl moiety on the immunological activity could be established.

## Results and Discussion

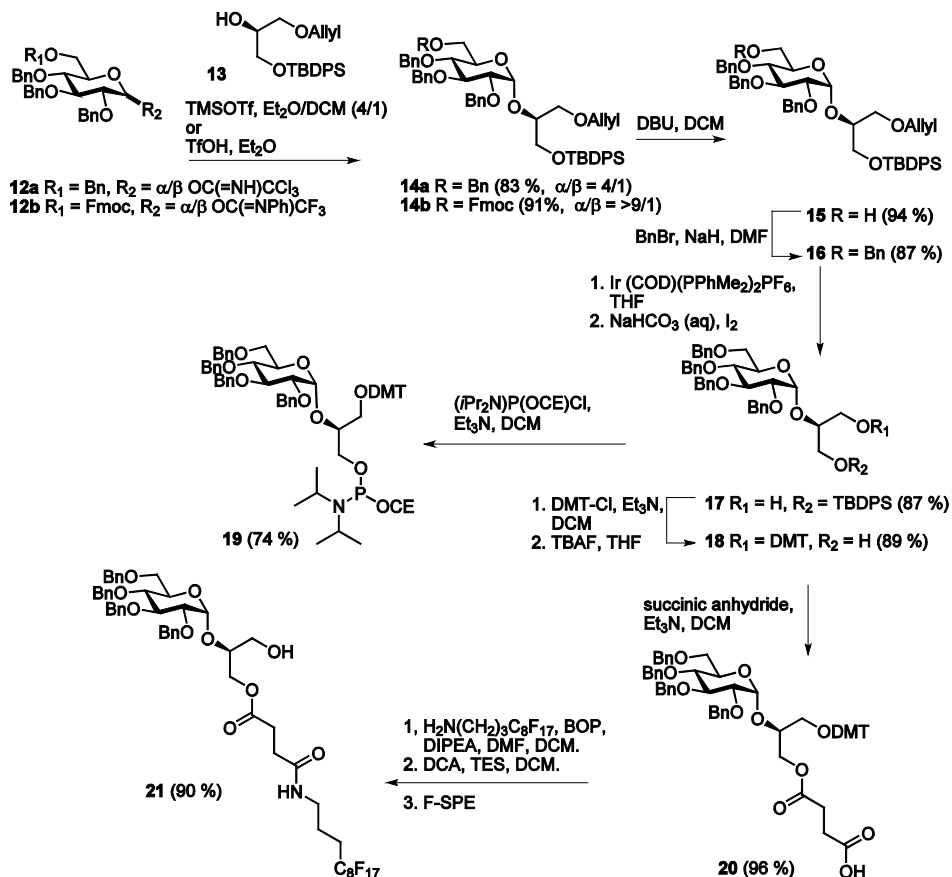
As described in **chapter four** perfluorooctylpropylsulfonyl ethanol (F-Pse) can be used effectively as a phosphate protecting group and concomitantly serve as a fluorous linker in the solution phase synthesis of TA fragments.<sup>11</sup> First the synthesis of hexamer **3**, a phosphorylated analogue of TA fragment **1**, was explored, using this

**Scheme 1.** Assembly of TA-fragment **3**.

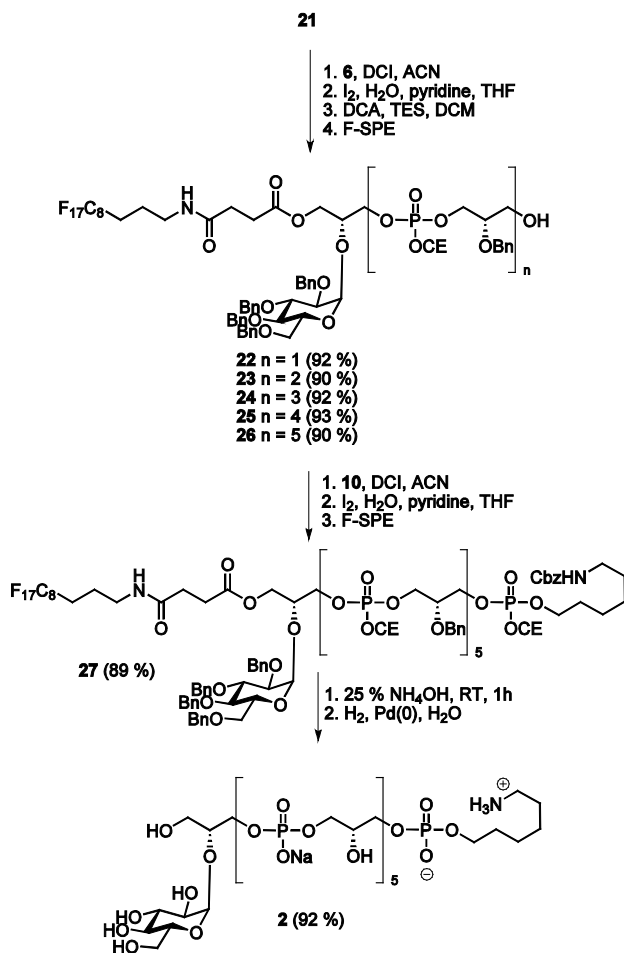


linker-system. Thus, F-Pse **5**<sup>13</sup> was elongated in a stepwise manner (Scheme 1) with glycerol phosphoramidite **6**<sup>14</sup> in an elongation process, which comprises coupling, oxidation, detritylation and, finally, a F-SPE<sup>7</sup> purification step. Before the F-SPE purification the crude reaction mixture was partitioned between acetonitrile/water (80/20) and hexane to remove the bulk of TES and DMT-H that are released during the detritylation step in order to simplify the F-SPE purification. Repeating this process four times led to pentamer **7** which was then subjected to an adapted elongation process. Coupling with benzylidene protected glucosylglycerol phosphoramidite **8**<sup>1</sup> under agency of DCI and standard oxidation was followed by detritylation using the milder PPTS/MeOH cocktail as the 4,6-*O*-benzylidene moiety is unstable towards DCA/TES.<sup>14,15</sup> The presence of the lipophilic protected carbohydrate moiety did not influence the F-SPE purification and the target compound was obtained uneventfully in 74% yield. At this stage an aminospacer was introduced to allow the conjugation of the target structure to, for instance, a carrier protein. Condensation of hexamer **9** and phosphoramidite **10** was followed by oxidation and F-SPE to give the fully protected construct **11** in 86% yield. Deprotection of hexamer **11** started by removal of the cyanoethyl (CE) and F-Pse groups by overnight treatment with aqueous ammonia at 40 °C. The semi-protected intermediate was separated from the released fluoros linker (perfluorooctylpropylsulfonylethyl amine) using a Et<sub>2</sub>O/H<sub>2</sub>O extraction. Subsequently, the benzylidene acetal, benzyl ethers and benzyl carbamate were removed by means of hydrogenolysis (Pd/H<sub>2</sub>), leading to the target hexamer **3** in 98% yield.

To attain the light fluoros assembly of TA fragments without a terminal phosphate moiety the next objective was to find a suitable fluoros hydroxyl protecting group. Inspired by contemporary DNA synthesis methods, a succinyl type linker was deemed suitable because of its stability towards phosphoramidite chemistry, oxidation and detritylation conditions.<sup>16,17</sup> The base lability of a fluoros succinyl linker allows the same deprotection strategy as employed in the synthesis of hexamer **3**. Moreover, attention was paid to the development of a more acid-stable glucosyl glycerol synthon that allows the incorporation of a glucosyl moiety at any stage of the elongation sequence. As described earlier, the benzylidene acetal does not withstand the standard detritylation conditions, necessitating the use of a carefully controlled procedure for the removal of the temporary DMT group. Therefore, the synthesis of the more acid stable tetra-*O*-benzyl glucosyl synthon **19** was undertaken (Scheme 2). A crucial step en route to synthon **19** is the stereoselective introduction of the  $\alpha$ -glucosidic linkage. First the use of per-benzylated glucosyl imidate **12a**<sup>18</sup> for the construction of this linkage was explored. Condensation of this donor with glycerol acceptor **13** in DCM led to formation of product **14a** with poor selectivity ( $\alpha/\beta = 2/1$ ). The use of ether as co-solvent<sup>19</sup> improved the  $\alpha/\beta$ -ratio (4/1), but the anomeric mixture proved to be inseparable. Next, a glucosyl donor bearing an Fmoc protecting group on the C6 hydroxyl known to favor the formation of the  $\alpha$ -product was

**Scheme 2.** Synthesis of phosphoramidite **19** and F-Pse linked glucosyl glycerol **21**.

explored.<sup>20-22</sup> Coupling 6-*O*-Fmoc glucosyl imidate **12b** with glycerol **13** using Et<sub>2</sub>O as a solvent, led to the formation of **14b** in high selectivity ( $\alpha/\beta \sim 10/1$ ). Purification by column chromatography, afforded  $\alpha$ -glucoside **14b** in 91% yield (containing < 3%  $\beta$ -adduct, based on <sup>1</sup>H-NMR analysis). Compound **14b** was then treated with DBU in DCM, and benzylation of the intermediate alcohol **15** led to tetrabenzylglucosyl derivative **16** in 87% yield. In the next step the allyl ether was removed by iridium catalyzed isomerisation, followed by oxidative cleavage of the intermediate enol ether, giving alcohol **17** in 87% yield. Installation of the DMT ether and desilylation led to building block **18**, which was transformed into the phosphoramidite synthon **19** using *N,N*-diisopropyl-2-cyanoethylchlorophosphoramidite and Et<sub>3</sub>N. Alternatively, **18** was reacted with succinic anhydride and Et<sub>3</sub>N in DCM, giving succinyl ester **20** in 96% yield (scheme 2). Coupling of **20** with perfluorooctylpropylamine, using BOP as a condensing agent, was followed by detritylation to give the crude fluorosyl glucosyl glycerol **21**, which was purified by F-SPE to give the pure target compound in 90%

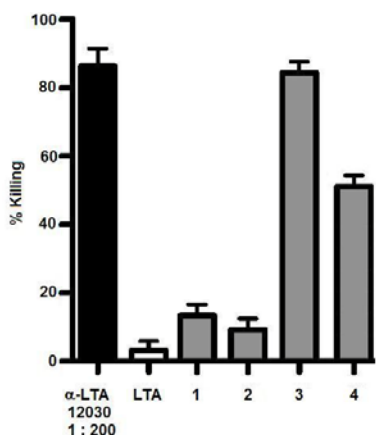
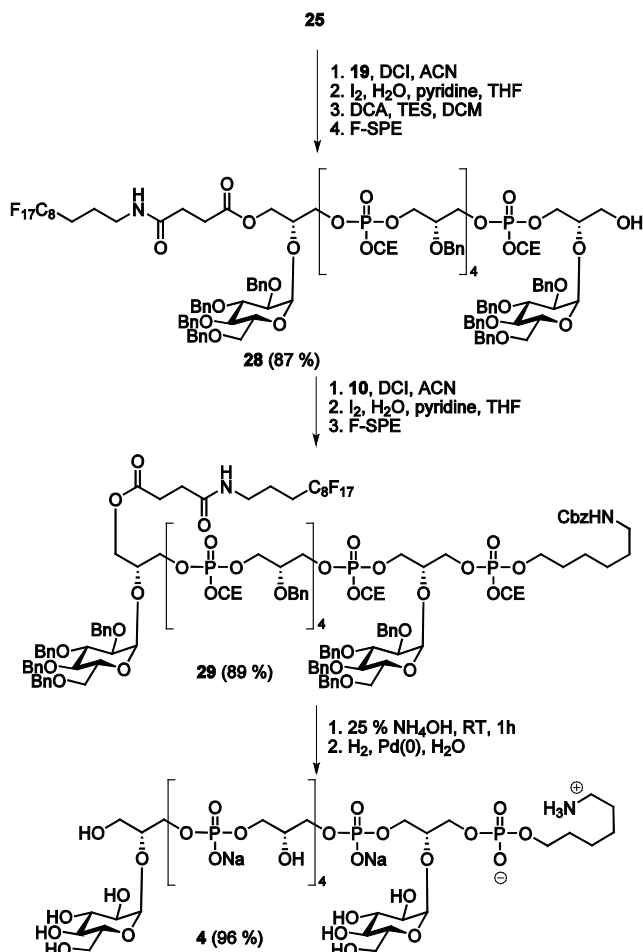
**Scheme 3.** Light fluororous assembly of **2**.

yield. This molecule was elongated in a step-wise manner with glycerol phosphoramidite **6** using a five-fold repetition of the 4-step elongation process described above leading to hexamer **26** (scheme 3). The aminohexyl-spacer was then introduced to give the fully protected hexamer **27**. Deprotection by 25% aqueous ammonia (1 hr, RT), was followed by hydrogenolysis to give 40 mg of target compound **2** (92%).

To broaden the palette of TA fragments, and further explore the effectiveness of the light fluororous chemistry, the synthesis was continued with the assembly of hexamer **4**, containing two glucosyl moieties (Scheme 4). Pentamer **25** was coupled to glucosylglycerol phosphoramidite **19**,

resulting in bis glucosylated hexamer **28** in 87%. Also this compound was uneventfully purified by F-SPE. After introduction of the spacer, the resulting hexamer **29** was deprotected using the aforementioned conditions, to yield the bis-glucosyl TA fragment **4** in 96% yield.

Hexameric TAs **3** and **4** were compared with TAs **1** and **2** on their ability to bind to rabbit antibodies raised against enterococcal LTA in an OPIA, that was performed as described before.<sup>1-4</sup> Surprisingly, TA **3** bearing an extra phosphate moiety compared to one of the lead fragments (**1**) showed no inhibitory activity at all, even when administered at a concentration of 400  $\mu g/ml$ . This indicates that the terminal phosphate moiety is at least disadvantageous to the immunogenicity of the TA. Compound **4**, which bears a second glucose moiety but lacks the terminal phosphate, showed some inhibitory potency. However, when compared to lead TA **2** the

**Scheme 4.** Light fluororous assembly of **29**.

of TA structures terminating in an alcohol functionality (structures **2** and **4**). Acid stable tetra-*O*-benzyl glucosyl building block **19**, allowing the incorporation of a glucosyl substitution at any position of the TA chain was

**Figure 2.** Hexamers **3,4** showing diminished inhibitory activity in the OPIA compared to previously made hexamers **1** and **2**. The left bar represents killing by the serum without addition of inhibitor. The second bar from the left (LTA) represents the positive control where native LTA is added as the inhibitor.

antibody binding was considerably reduced, indicating that an extra glucosyl moiety has a detrimental effect on antigenicity (see figure 2).

## Conclusion

In summary, this chapter describes the development of two complementary fluororous linker systems for the assembly of glucosylated TA fragments. The first linker, perfluorooctylpropyl-sulfonyl-ethyl, is used as a phosphate protecting group and allows the assembly of TA fragments featuring a terminal phosphate monoester (hexamer **3**). The second linker, a perfluorooctyl-propyl succinyl system, is used as a hydroxyl protecting functionality and leads to the formation

prepared and applied in the assembly of TAs **1** and **2**. The presence of two lipophilic tetrabenzylglucosyl moieties in the fully protected precursor (**29**) of **4** did not have a negative effect on the F-SPE purifications. Fluorous chemistry is an efficient means for the assembly of (glycosylated) TA fragments and allows the construction of pure TA oligomers in multi milligram quantities, sufficient for most initial biochemical studies.

## Experimental section

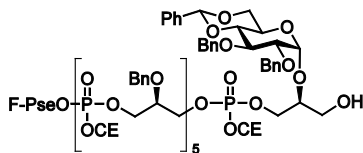
**General Procedures and Material:** All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma-Aldrich, Genscript, Fluorous Technologies) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H<sub>2</sub>SO<sub>4</sub> in ethanol or with a solution of (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>•4 H<sub>2</sub>O 25 g/l and (NH<sub>4</sub>)<sub>4</sub>Ce(SO<sub>4</sub>)<sub>4</sub>•2 H<sub>2</sub>O 10 g/l, in 10% aqueous H<sub>2</sub>SO<sub>4</sub> followed by charring at +/- 140 °C. Some unsaturated compounds were visualized by spraying with a solution of KMnO<sub>4</sub> (2%) and K<sub>2</sub>CO<sub>3</sub> (1%) in water. Optical rotation measurements ([α]<sub>D</sub><sup>20</sup>) were performed on a Propol automated polarimeter (Sodium D-line, λ = 589 nm) with a concentration of 10 mg/ml (c = 1), unless stated otherwise. Infrared spectra were recorded on a Shimadzu FT-IR 8300. <sup>31</sup>P, <sup>1</sup>H, and <sup>13</sup>C NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in CDCl<sub>3</sub> with chemical shift (δ) relative to tetramethylsilane, unless stated otherwise. When D<sub>2</sub>O was used, <sup>1</sup>H-NMR spectra were recorded with chemical shift relative (δ) to HDO (4.755 ppm), <sup>31</sup>P spectra were measured with chemical shift relative to 85% H<sub>3</sub>PO<sub>4</sub> (external standard) and <sup>13</sup>C-NMR spectra were recorded with chemical shift relative to TMS (external standard). High resolution mass spectra (HRMS) were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and either 0.1% formic acid or 10mM ammonium formate for the oligomers) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

**General procedure for phosphoramidite coupling, oxidation, detritylation and FSPE on a typical scale (0.1-0.25 mmol):** Starting alcohol was dissolved in ACN (0.1M). DCI (0.25M solution in CH<sub>3</sub>CN, 2 eq compared to phosphoramidite) was added, together with freshly activated MS3Å and the mixture was stirred under argon for 15 minutes. Phosphoramidite (0.175M in ACN, 1.3 - 4.0 eq) was added and the reaction was stirred until TLC analysis revealed full conversion of the starting material into a higher running spot (~1 hr). Added were, respectively, H<sub>2</sub>O (~1 ml) and I<sub>2</sub> (0.2 M in THF/pyr 4/1), and the mixture was stirred for an additional 5 min. The mixture was diluted with EtOAc (~50 ml) and washed with, respectively, sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (~20 ml), 0.5 M KHSO<sub>4</sub> (~20 ml) and a 1/1 mixture of sat. aq. NaHCO<sub>3</sub> and brine (~20 ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> (s) and concentrated under reduced pressure. The residue was coevaporated once with toluene (10 ml) before it was redissolved in DCM. Triethylsilane and dichloroacetic acid were added and the mixture was stirred until the bright orange color fully disappeared (~30 min). DCM (~40 ml) was added and the organic layer was washed with a 1/1 mixture of sat. aq. NaHCO<sub>3</sub> and brine (~20 ml, check if pH >7), before it was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. The residue was taken up in 4/1



ACN/H<sub>2</sub>O (10 ml) and washed with hexane (50 ml). The hexane layer was extracted twice with 4/1 ACN/H<sub>2</sub>O (2 x 10 ml) and the combined ACN/H<sub>2</sub>O layers were concentrated under reduced pressure in a 100 ml pear shaped flask. The residue was taken up in 0.5 ml ACN and applied to a small column containing fluoroflash<sup>TM</sup> fluorosilica (4g) which was preeluted with 1/1 ACN H<sub>2</sub>O. The column was eluted with 1/1 ACN/H<sub>2</sub>O until all the non-fluorous byproducts (DMT-H, phosphates, DCI) were removed. Subsequently the fluorosilica product was eluted from the column with, respectively, CH<sub>3</sub>CN and acetone.

**Global deprotection and purification of oligomers:** The fully protected oligomer was treated with a 9/1 mixture of 28% NH<sub>4</sub>OH (aq)/1,4-dioxane at a concentration of 5 mg/ml at 40 - 45 °C overnight in a sealed flask or tube in case of **11**. In the synthesis of oligomers **2** and **4**, the corresponding protected hexamers (**27** and **29**, respectively) were treated with a 9/1 mixture of 28% NH<sub>4</sub>OH (aq)/1,4-dioxane at a concentration of 5 mg/ml at room temperature for 1h. Next, in all cases, the mixture was washed with Et<sub>2</sub>O (equal volume) and the ether layer was extracted twice with H<sub>2</sub>O. The aqueous layer was concentrated under reduced pressure after which NMR and HRMS analysis confirmed full conversion to the semiprotected intermediate. The intermediate was then treated with Pd (0)/H<sub>2</sub> in a slightly acidic (pH ~2.7) mixture of dioxane/water (1/4, containing ~1% AcOH). After stirring for three days the mixture was filtered and concentrated *in vacuo*. The residue was purified by size exclusion chromatography (Sephadex HW40, eluent: 0.15 M NH<sub>4</sub>OAc). After repeated lyophilisation, the purified product was eluted through a small column containing Dowex Na<sup>+</sup> cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H<sub>2</sub>O, flushed with H<sub>2</sub>O and MeOH before use). Lyophilization gave the fully deprotected oligomer of which the integrity and purity was confirmed by HRMS and NMR (<sup>1</sup>H, <sup>13</sup>C, <sup>31</sup>P) analysis.

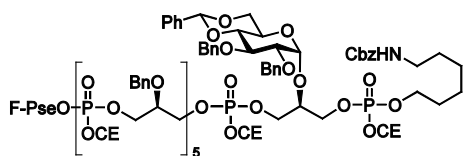


#### Glucosyl-2-O-benzylglycerol phosphate hexamer (**9**)

Glycerol phosphate pentamer **7** (286 mg, 139 μmol) and DCI (0.25M solution in CH<sub>3</sub>CN, 2.22 ml, 556 μmol) were dissolved in CH<sub>3</sub>CN (2.0 ml) together with freshly activated MS3Å and stirred for 15 min under argon. Subsequently, glucosyl-glycerol phosphoramidite **8** (0.1M in CH<sub>3</sub>CN, 2.30 ml, 230 μmol) was added and the mixture

stirred for 30 min at RT. H<sub>2</sub>O (1.0 ml) was added after which the oxidation step was performed according to the general procedure. The crude intermediate was redissolved in a 1/1 mixture of DCM and MeOH (40 ml) and treated with PPTS (40 mg, 0.16 mmol) for 8 hrs under gentle stirring. The mixture was diluted with DCM (80 ml) and washed with a 1/1 mixture of sat. aq. NaHCO<sub>3</sub> and brine (50 ml). The organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*, after which the crude product was purified with FSPE, according to the general procedure. Glucosylated hexamer **9** (277 mg, 103 μmol, 74%) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.8 (1P), -1.3 (2P), -1.1 - -0.9 (3P); <sup>1</sup>H NMR (400 MHz): δ = 2.09 - 2.35 (m, 4H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 2.47 - 2.77 (m, 13H, 6 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>-OH), 3.05 - 3.13 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.24 - 3.34 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.51 - 3.72 (m, 5H, H-2, H-4, H-6, CH<sub>2</sub> glycerol), 3.77 - 3.88 (m, 6H, 6 x CH glycerol), 3.98 - 4.34 (m, 37H, H-3, H-5, H-6', 11 x CH<sub>2</sub> glycerol, 6 x CH<sub>2</sub> cyanoethyl), 4.42 - 4.50 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 4.56 - 4.65 (m, 10H, 5 x CH<sub>2</sub> Bn), 4.70 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.80 (d, 1H, *J* = 11.3 Hz, CHH Bn), 4.87 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.91 (d, 1H, *J* = 3.7 Hz, H-1), 4.95 (d, 1H, *J* = 11.3 Hz, CHH Bn), 5.55 (s, 1H, CH benzylidene), 7.25 - 7.40 (m, 38H, H<sub>arom</sub>), 7.44 - 7.48 (m, 2H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 13.3 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 19.2 - 19.5 (12 x CH<sub>2</sub> cyanoethyl), 29.2 (t, *J* = 22 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 53.0, 53.1 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 60.9 (CH<sub>2</sub> glycerol), 61.3 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 62.0 - 62.4 (6 x CH<sub>2</sub> cyanoethyl), 62.8 (C-5), 65.4 - 66.0 (10 x CH<sub>2</sub> glycerol),

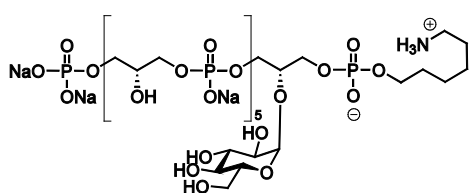
67.2 (CH<sub>2</sub> glycerol), 68.7 (C-6), 72.0 - 72.1 (5 x CH<sub>2</sub> Bn), 74.3 (CH<sub>2</sub> Bn), 75.0 (CH<sub>2</sub> Bn), 75.2 - 75.4 (5 x CH glycerol), 78.6 - 78.8 (C-2, C-3, CH glycerol), 82.0 (C-4), 98.6, 98.9 (C-1), 100.9 (CH benzylidene), 116.6 - 116.7 (6 x C<sub>q</sub> cyanoethyl), 125.7 (CH<sub>arom</sub>), 127.6 - 128.9 (CH<sub>arom</sub>), 137.1 - 137.4 (7 x C<sub>q</sub> Bn), 138.4 (C<sub>q</sub> benzylidene); HRMS: C<sub>111</sub>H<sub>127</sub>F<sub>17</sub>N<sub>6</sub>O<sub>38</sub>P<sub>6</sub>S + NH<sub>4</sub><sup>+</sup> requires 2710.6403, found 2710.6393.



### Glucosyl-2-O-benzylglycerol phosphate hexamer aminoethyl spacer (11)

Hexamer **9** (272 mg, 101 μmol) was coupled to spacer phosphoramidite **10** (4 eq), oxidized and purified (FSPE) using the general procedure as described above. Fully protected hexamer **11** (267 mg, 87.2 μmol, 86%) was

obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.9, -1.8 (1P), -1.3 - -1.0 (6P); <sup>1</sup>H NMR (400 MHz): δ = 1.27 - 1.37 (m, 4H, 2 x CH<sub>2</sub> hexylspacer), 1.42 - 1.51 (m, 2H, CH<sub>2</sub> hexylspacer), 1.60 - 1.68 (m, 2H, CH<sub>2</sub> hexylspacer), 2.09 - 2.34 (m, 4H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 2.39 - 2.69 (m, 14H, 7 x CH<sub>2</sub> cyanoethyl), 3.05 - 3.17 (m, 4H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub>-N hexylspacer), 3.24 - 3.33 (m, 2H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 3.57 - 3.72 (m, 3H, H-2, H-4, H-6), 3.75 - 3.85 (m, 5H, 5 x CH glycerol), 3.94 - 4.33 (m, 44H, H-3, H-5, H-6', CH glycerol, 12 x CH<sub>2</sub> glycerol, 7 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>-O hexylspacer), 4.43 - 4.50 (m, 4H, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, CH<sub>2</sub> Bn), 4.53 - 4.64 (m, 10H, 5 x CH<sub>2</sub> Bn), 4.71 - 4.77 (m, 2H, CH<sub>2</sub> Bn), 4.81, (d, 1H, J = 11.6 Hz, CHH Bn), 4.92, (d, 1H, J = 11.6 Hz, CHH Bn), 4.99 - 5.12 (m, 4H, H-1, NH CBz, CH<sub>2</sub> CBz), 5.55 (s, 1H, CH benzylidene), 7.26 - 7.38 (m, 43H, H<sub>arom</sub>), 7.44 - 7.48 (m, 2H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 13.3 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 19.2 - 19.5 (7 x CH<sub>2</sub> cyanoethyl), 24.8, 25.9 (2 x CH<sub>2</sub> hexylspacer), 29.3 - 29.9 (2 x CH<sub>2</sub> hexylspacer, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 40.7 (CH<sub>2</sub>-N hexylspacer), 53.0, 53.1 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-, -OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 61.3 (-OCH<sub>2</sub>CH<sub>2</sub>SO<sub>2</sub>-), 61.8 - 62.4 (7 x CH<sub>2</sub> cyanoethyl), 62.8 (C-5), 65.3 - 66.0 (11 x CH<sub>2</sub> glycerol), 66.3 (CH<sub>2</sub> CBz), 68.4 - 68.6 (C-6, CH<sub>2</sub> glycerol), 72.0 - 72.2 (5 x CH<sub>2</sub> Bn), 73.4 - 73.5 (CH<sub>2</sub> Bn), 75.0 (CH<sub>2</sub> Bn), 75.2 - 75.5 (6 x CH glycerol), 78.0 - 78.1 (C-3), 78.9 (C-2), 81.7 - 81.8 (C-4), 97.4 - 97.7 (C-1), 100.8 (CH benzylidene), 116.6 - 116.7 (7 x C<sub>q</sub> cyanoethyl), 125.7 (CH<sub>arom</sub>), 127.5 - 128.9 (CH<sub>arom</sub>), 136.6 (C<sub>q</sub> Bn), 137.1 - 137.3 (6 x C<sub>q</sub> Bn), 137.9 (C<sub>q</sub> Bn), 138.5 (C<sub>q</sub> benzylidene), 156.3 (C<sub>q</sub> CBz); HRMS: C<sub>128</sub>H<sub>150</sub>F<sub>17</sub>N<sub>8</sub>O<sub>43</sub>P<sub>7</sub>S + NH<sub>4</sub><sup>+</sup> requires 3076.7748, found 3076.7789.

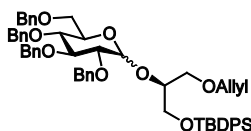


### Glucosyl-glycerolphosphate hexamer (3)

Protected hexamer **11** (99.5 mg, 32.5 μmol) was treated with aqueous ammonia as described above. Additionally, the compound was eluted through a small column containing Dowex Na<sup>+</sup> cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H<sub>2</sub>O, flushed with H<sub>2</sub>O and MeOH before use) and,

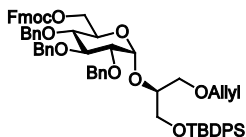
subsequently, lyophilized, yielding the intermediate semiprotected hexamer (75.2 mg, 32.5 μmol, 100%) as an amorphous white solid. Analytical data intermediate: <sup>31</sup>P NMR (161.7 MHz, D<sub>2</sub>O): δ = 0.9 - 1.1 (6P), 2.9 (1P, phosphomonoester); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ = 0.95 - 1.24 (m, 6H, 3 x CH<sub>2</sub> hexylspacer), 1.34 - 1.44 (m, 2H, CH<sub>2</sub> hexylspacer), 2.85 - 2.94 (m, 2H, CH<sub>2</sub>-N hexylspacer), 3.51 - 4.15 (m, 38H, H-2, H-3, H-4, H-5, H-6, H-6', CH<sub>2</sub>-O hexylspacer, 6 x CH glycerol, 12 x CH<sub>2</sub> glycerol), 4.29 - 4.41 (m, 10H, 5 x CH<sub>2</sub> Bn), 4.47 - 4.58 (m, 4H, 2 x CH<sub>2</sub> Bn), 4.89 (s, 2H, CH<sub>2</sub> CBz), 5.29 (d, 1H, J = 3.6 Hz, H-1), 5.47 (s, 1H, CH benzylidene), 6.98 - 7.37 (m, 45H, H<sub>arom</sub>); HRMS: [C<sub>94</sub>H<sub>120</sub>NO<sub>41</sub>P<sub>7</sub> + 2H]<sup>2+</sup> requires 1068.7822, found 1068.7828. A portion of the intermediate (75.1 mg, 32.5 μmol) was deprotected with Pd (0)/H<sub>2</sub> using the standard

procedure. Monoglucosylated hexamer **3** (45.5 mg, 31.7  $\mu$ mol, 98%) was obtained as an amorphous white solid.  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 0.9 (1P), 1.2 - 1.3 (4P), 1.4 (1P), 4.7 (1P, phosphomonoester);  $^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.38 - 1.43 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.60 - 1.68 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.97 (t, 2H,  $J$  = 7.5 Hz,  $\text{CH}_2$ -N hexylspacer), 3.37 (at, 1H,  $J$  = 9.6 Hz, H-4), 3.49 (dd, 1H,  $J$  = 3.8 Hz, 9.9 Hz, H-2), 3.71 - 3.77 (m, 3H, H-3, H-6,  $\text{CHH}$  glycerol), 3.79 - 4.04 (m, 32H, H-5, H-6', 5 x CH glycerol, 11 x  $\text{CH}_2$  glycerol,  $\text{CHH}$  glycerol,  $\text{CH}_2$ -O hexylspacer), 4.06 - 4.09 (m, 1H, CH glycerol), 5.14 (d, 1H,  $J$  = 3.7 Hz, H-1);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 25.4, 26.1, 27.6 (3 x  $\text{CH}_2$  hexylspacer), 30.4 (d,  $J$  = 6.8 Hz,  $\text{CH}_2$  hexylspacer), 40.4 ( $\text{CH}_2$ -N hexylspacer), 61.5 (C-6), 65.2 (d,  $J$  = 6.0 Hz,  $\text{CH}_2$  glycerol), 65.7 (d,  $J$  = 4.5 Hz,  $\text{CH}_2$  glycerol), 66.1 (d,  $J$  = 5.2 Hz,  $\text{CH}_2$  glycerol), 67.1 - 67.3 (8 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$ -O hexylspacer), 67.7 (d,  $J$  = 5.5 Hz,  $\text{CH}_2$  glycerol), 70.5 (t,  $J$  = 7.7 Hz, 4 x CH glycerol), 70.7 (C-4), 71.3 (t,  $J$  = 7.3 Hz, CH glycerol), 72.5 (C-2), 72.8 (C-5), 73.9 (C-3), 76.4 (t,  $J$  = 8.0 Hz, CH glycerol), 98.7 (C-1); HRMS:  $\text{C}_{30}\text{H}_{68}\text{NO}_{39}\text{P}_7 + \text{H}^+$  requires 1284.1605, found 1284.1610.



### 3-O-Allyl-2-O-(2,3,4,6-tetra-O-benzyl- $\alpha,\beta$ -D-glucopyranosyl)-1-O-(tert-butylidiphenylsilyl)-sn-glycerol (**14a**)

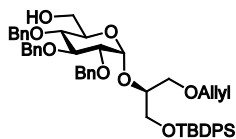
To a cooled (0  $^\circ\text{C}$ ) solution of donor **12a** (171 mg, 0.250 mmol) and semiprotected glycerol **13** (111 mg, 0.300 mmol) in a 4/1 mixture of  $\text{Et}_2\text{O}$ /DCM (5.0 ml) was added TMSOTf (2.25  $\mu\text{l}$ , 12.4  $\mu\text{mol}$ ). After stirring 40 min,  $\text{Et}_3\text{N}$  (3 drops) was added and the mixture diluted with DCM (10 ml). After washing once with a 1/1 mixture of sat. aq.  $\text{NaHCO}_3$  and brine (10 ml), the organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography ( $\text{EtOAc}$ /PE) gave pseudodisaccharide **14a** (168 mg, 0.188 mmol, 75%) as an inseparable mixture of anomers ( $\alpha/\beta$  ratio of  $\sim 4/1$ , based on  $^1\text{H}$ -NMR analysis. This was  $\sim 2/1$  when the reaction was performed in pure DCM). For analytical data of the pure  $\alpha$ -isomer see the synthesis of compound **16**.



### 3-O-Allyl-2-O-(2,3,4-tri-O-benzyl-6-O-[9-fluorenylmethyloxycarbonyl]- $\alpha$ -D-glucopyranosyl)-1-O-(tert-butylidiphenylsilyl)-sn-glycerol (**14b**)

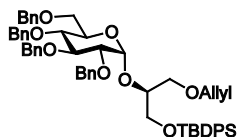
To a cooled (0  $^\circ\text{C}$ ) solution of donor **12b** (7.51 g, 8.90 mmol) and semiprotected glycerol **13** (3.96 g, 10.7 mmol) in  $\text{Et}_2\text{O}$  (180 ml) was added TfOH (157  $\mu\text{l}$ , 1.78 mmol). After stirring 25 min, sat. aq.  $\text{NaHCO}_3$  (75 ml) was added and the layers separated. The ether layer was washed once with brine (50 ml) before it was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography ( $\text{EtOAc}$ /PE) gave pseudodisaccharide **14b** (8.30 g, 8.09 mmol, 91%) as a colourless oil containing a minor amount ( $< 3\%$ , based on  $^1\text{H}$ -NMR analysis) of the  $\beta$ -product.  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): +32.0; IR: 1007, 1072, 1254, 1450, 1748, 2859;  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.05 (s, 9H, *t*-Bu TBDPS), 3.58 - 3.63 (m, 3H, H-2, H-4,  $\text{CHH}$  glycerol), 3.69 - 3.80 (m, 3H,  $\text{CHH}$  glycerol,  $\text{CH}_2$  glycerol), 3.94 - 4.14 (m, 6H, H-3, H-5, CH glycerol,  $\text{CHH}$  Fmoc,  $\text{CH}_2$  allyl), 4.20 - 4.26 (m, 2H,  $\text{CHH}$  Fmoc, CH Fmoc), 4.31 - 4.40 (m, 2H, H-6, H-6'), 4.55 (d, 1H,  $J$  = 10.8 Hz,  $\text{CHH}$  Bn), 4.69 (d, 1H,  $J$  = 11.6 Hz,  $\text{CHH}$  Bn), 4.76 - 4.79 (m, 3H, 1 x  $\text{CH}_2$  Bn,  $\text{CHH}$  Bn), 4.88 (d, 1H,  $J$  = 10.8 Hz,  $\text{CHH}$  Bn), 5.00 (d, 1H,  $J$  = 10.8 Hz,  $\text{CHH}$  Bn), 5.16 (ad, 1H,  $J$  = 10.8 Hz,  $\text{CHH}$  allyl), 5.25 (dd, 1H,  $J$  = 1.4 Hz, 17.4 Hz,  $\text{CHH}$  allyl), 5.32 (d, 1H,  $J$  = 3.6 Hz, H-1), 5.87 (ddd, 1H,  $J$  = 5.5 Hz, 10.7 Hz, 22.6 Hz, CH allyl), 7.22 - 7.40 (m, 25H,  $\text{H}_{\text{arom}}$ ), 7.58 (d, 1H,  $J$  = 7.5 Hz,  $\text{H}_{\text{arom}}$ ), 7.61 (d, 1H,  $J$  = 7.5 Hz,  $\text{H}_{\text{arom}}$ ), 7.66 (d, 4H,  $J$  = 7.1 Hz,  $\text{H}_{\text{arom}}$ ), 7.75 (d, 2H,  $J$  = 7.6 Hz,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 19.2 ( $\text{C}_q$  *t*-Bu), 26.8 (3 x  $\text{CH}_3$  TBDPS), 46.6 (CH Fmoc), 63.8 ( $\text{CH}_2$  glycerol), 66.2 ( $\text{CH}_2$  Fmoc), 68.5 (C-5), 69.9 (C-6), 70.6 ( $\text{CH}_2$  glycerol), 72.2 ( $\text{CH}_2$  allyl), 72.3 ( $\text{CH}_2$  Bn), 75.0 ( $\text{CH}_2$  Bn), 75.7 ( $\text{CH}_2$  Bn), 75.8 (CH glycerol), 77.1 (C-4), 79.5 (C-2), 81.7 (C-3), 95.7 (C-1), 116.9 ( $\text{CH}_2$  allyl), 125.1, 125.2 ( $\text{CH}_{\text{arom}}$ ), 127.1 - 128.6 ( $\text{CH}_{\text{arom}}$ ), 129.7 ( $\text{CH}_{\text{arom}}$ ), 133.1, 133.2 ( $\text{C}_q$  phenyl), 134.6 (CH

allyl), 135.5 ( $\text{CH}_{\text{arom}}$ ), 138.1, 138.2, 138.7, 138.8 (3 x  $\text{C}_q$  Bn), 141.2, 141.2 (2 x  $\text{C}_q$  FMOC), 143.2, 143.4 (2 x  $\text{C}_q$  FMOC), 155.0 ( $\text{C}=\text{O}$  FMOC); HRMS:  $\text{C}_{64}\text{H}_{68}\text{O}_{10}\text{Si} + \text{NH}_4^+$  requires 1042.4920, found 1042.4933.



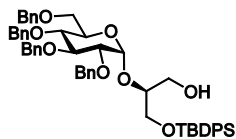
### 3-O-Allyl-2-O-(2,3,4-tri-O-benzyl- $\alpha$ -D-glucopyranosyl)-1-O-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**15**)

To a solution of compound **14b** (3.40 g, 3.32 mmol) in DCM (65 ml) was added DBU (165  $\mu\text{l}$ , 1.10 mmol). After stirring for 15 min the solvent was removed under reduced pressure and the residue purified by silica gel column chromatography ( $\text{EtOAc/PE}$ ) giving alcohol **15** (2.50 g, 3.11 mmol, 94%) as a colourless oil.  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): +33.6; IR: 737, 1026, 1072, 1454, 2928;  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.03 (s, 9H, *t*-Bu TBDPS), 3.48 - 3.80 (m, 9H, H-2, H-4, H-5, H-6, H-6', 2 x  $\text{CH}_2$  glycerol), 3.97 - 4.04 (m, 4H, H-3, CH glycerol,  $\text{CH}_2$  allyl), 4.61 (d, 1H,  $J$  = 10.8 Hz, CHH Bn), 4.68 (d, 1H,  $J$  = 12.0 Hz, CHH Bn), 4.76 (d, 1H,  $J$  = 12.0 Hz, CHH Bn), 4.78 (d, 1H,  $J$  = 10.4 Hz, CHH Bn), 4.86 (d, 1H,  $J$  = 11.2 Hz, CHH Bn), 4.97 (d, 1H,  $J$  = 10.8 Hz, CHH Bn), 5.16 (dd, 1H,  $J$  = 1.6 Hz, 10.4 Hz, CHH allyl), 5.24 - 5.28 (m, 2H, H-1, CHH allyl), 5.88 (ddd, 1H,  $J$  = 5.5 Hz, 10.7 Hz, 22.6 Hz, CH allyl), 7.24 - 7.42 (m, 21H,  $\text{H}_{\text{arom}}$ ), 7.64 - 7.67 (m, 4H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 19.1 ( $\text{C}_q$  *t*-Bu), 26.8 (3 x  $\text{CH}_3$  TBDPS), 61.4 (C-6), 63.8 ( $\text{CH}_2$  glycerol), 70.7 (C-5), 70.9 ( $\text{CH}_2$  glycerol), 72.2 ( $\text{CH}_2$  allyl), 72.3 ( $\text{CH}_2$  Bn), 74.9 ( $\text{CH}_2$  Bn), 75.6 ( $\text{CH}_2$  Bn), 76.0 (CH glycerol), 77.1 (C-4), 79.6 (C-2), 81.6 (C-3), 96.0 (C-1), 116.9 ( $\text{CH}_2$  allyl), 127.5 - 128.4 ( $\text{CH}_{\text{arom}}$ ), 129.7 ( $\text{CH}_{\text{arom}}$ ), 133.1, 133.2 ( $\text{C}_q$  phenyl), 134.6 (CH allyl), 135.5 ( $\text{CH}_{\text{arom}}$ ), 138.2, 138.3, 138.8 (3 x  $\text{C}_q$  Bn); HRMS:  $\text{C}_{49}\text{H}_{58}\text{O}_8\text{Si} + \text{Na}^+$  requires 825.3793, found 825.3784.



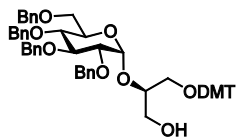
### 3-O-Allyl-2-O-(2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl)-1-O-(*tert*-butyldiphenylsilyl)-*sn*-glycerol (**16**)

A solution of alcohol **15** (2.521 g, 3.14 mmol) together with BnBr (0.94 ml, 7.85 mmol) in DMF (20 ml) was stirred for 5 minutes at 0  $^\circ\text{C}$ , after which NaH (60% dispersion in mineral oil, 0.314 g, 7.85 mmol) was added. The resulting mixture was stirred for 75 min and allowed to slowly warm up to RT, before MeOH (5.0 ml) was added. After stirring for 15 min,  $\text{H}_2\text{O}$  (30 ml) was added and the mixture extracted with  $\text{Et}_2\text{O}$  (50 ml). The organic layer was washed twice with  $\text{H}_2\text{O}$  (20 ml) and once with brine (20 ml) before it was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo*. Purification of the residual oil by silica gel column chromatography ( $\text{EtOAc/PE}$ ) furnished perbenzylglucosyl glycerol derivative **16** (2.429 g, 2.72 mmol, 87%) as a colourless oil.  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): +31.8; IR: 737, 1026, 1069, 1454, 2928;  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.05 (s, 9H, *t*-Bu TBDPS), 3.36 (dd, 1H,  $J$  = 1.7 Hz, 10.6 Hz, H-6), 3.54 - 3.84 (m, 8H, H-2, H-4, H-5, H-6', 2 x  $\text{CH}_2$  gly), 3.95 - 4.00 (m, 3H,  $\text{CH}_2$  allyl, H-3), 4.06 (m, 1H, CH glycerol), 4.36 (d, 1H,  $J$  = 12.4 Hz, CHH Bn), 4.43 (d, 1H,  $J$  = 10.8 Hz, CHH Bn), 4.55 (d, 1H,  $J$  = 12.0 Hz, CHH Bn), 4.69 (d, 1H,  $J$  = 12.0 Hz, CHH Bn), 4.74 - 4.82 (m, 3H,  $\text{CH}_2$  Bn, CHH Bn), 4.97 (d, 1H,  $J$  = 10.8 Hz, CHH Bn), 5.15 (dd, 1H,  $J$  = 1.4 Hz, 10.6 Hz, CHH allyl), 5.24 - 5.29 (m, 2H, CHH allyl, H-1), 5.88 (ddd, 1H,  $J$  = 5.5 Hz, 10.7 Hz, 22.7 Hz, CH allyl), 7.08 - 7.11 (m, 2H,  $\text{H}_{\text{arom}}$ ), 7.20 - 7.38 (m, 24H,  $\text{H}_{\text{arom}}$ ), 7.64 - 7.67 (m, 4H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 19.1 ( $\text{C}_q$  *t*-Bu), 26.8 (3 x  $\text{CH}_3$  TBDPS), 63.8 ( $\text{CH}_2$  glycerol), 68.0 (C-6), 70.1 (C-5), 70.5 ( $\text{CH}_2$  glycerol), 72.2 ( $\text{CH}_2$  allyl), 72.2 ( $\text{CH}_2$  Bn), 73.3 ( $\text{CH}_2$  Bn), 74.8 ( $\text{CH}_2$  Bn), 75.5 ( $\text{CH}_2$  Bn), 76.0 (CH glycerol), 77.4 (C-4), 79.5 (C-2), 81.8 (C-3), 96.1 (C-1), 116.7 ( $\text{CH}_2$  allyl), 127.4 - 128.2 ( $\text{CH}_{\text{arom}}$ ), 129.6 ( $\text{CH}_{\text{arom}}$ ), 133.1, 133.3 ( $\text{C}_q$  phenyl), 134.6 (CH allyl), 135.5 ( $\text{CH}_{\text{arom}}$ ), 137.9, 138.3, 138.4, 138.8 (4 x  $\text{C}_q$  Bn); HRMS:  $\text{C}_{56}\text{H}_{64}\text{O}_8\text{Si} + \text{NH}_4^+$  requires 910.4709, found 910.4718.



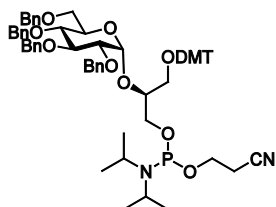
**2-O-(2,3,4,6-tetra-O-Benzyl-α-D-glucopyranosyl)-1-O-(tert-butylidiphenylsilyl)-sn-glycerol (17)**

A solution of glycoside **16** (2.37 g, 2.65 mmol) in freshly distilled THF (18 ml) was stirred under argon for 30 min. After the addition of Ir(COD)(Ph<sub>2</sub>MeP)<sub>2</sub>PF<sub>6</sub> (112 mg, 0.133 mmol) the solution was purged with H<sub>2</sub> (g) for ~15s. After stirring under argon for 2 hrs, the mixture was diluted with THF (20 ml) and sat. aq. NaHCO<sub>3</sub> (20 ml). Upon addition of I<sub>2</sub> (1.01 g, 3.98 mmol), the mixture was allowed to stir for 1.5 hrs at room temperature. The mixture was then diluted with EtOAc (100 ml) and washed with, respectively, sat. aq. Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (30 ml) and brine (40 ml). The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Column chromatography (EtOAc/PE) afforded **17** (1.97 g, 2.31 mmol, 87%) as a colourless oil. [α]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +21.4; IR: 737, 1026, 1069, 1454, 1724, 2928, 3449; <sup>1</sup>H NMR (400 MHz): δ = 1.05 (s, 9H, *t*-Bu TBDPS), 3.11 (bs, 1H, CH<sub>2</sub>OH), 3.32 (dd, 1H, *J* = 1.5 Hz, 10.6 Hz, H-6), 3.54 - 3.57 (m, 2H, H-2, H-6'), 3.62 - 3.70 (m, 3H, H-4, 2 x CHH glycerol), 3.77 - 3.86 (m, 4H, H-5, CH glycerol, 2 x CHH glycerol), 3.97 (t, 1H, *J* = 9.3 Hz, H-3), 4.33 (d, 1H, *J* = 12.2 Hz, CHH Bn), 4.44 (d, 1H, *J* = 10.9 Hz, CHH Bn), 4.52 (d, 1H, *J* = 12.2 Hz, CHH Bn), 4.66 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.77 - 4.85 (m, 3H, CH<sub>2</sub> Bn, CHH Bn), 4.90 - 4.93 (m, 2H, H-1, CHH Bn), 7.08 - 7.12 (m, 2H, H<sub>arom</sub>), 7.20 - 7.39 (m, 24H, H<sub>arom</sub>), 7.62 - 7.64 (m, 4H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 19.0 (C<sub>q</sub> *t*-Bu), 26.7 (3 x CH<sub>3</sub> TBDPS), 62.6 (CH<sub>2</sub> glycerol), 63.7 (CH<sub>2</sub> glycerol), 67.8 (C-6), 70.4 (C-5), 73.3, 73.8, 74.7, 75.5 (4 x CH<sub>2</sub> Bn), 77.4 (C-4), 79.4 (C-2), 80.8 (CH glycerol), 82.0 (C-3), 98.4 (C-1), 127.4 - 128.4 (CH<sub>arom</sub>), 129.6 (CH<sub>arom</sub>), 132.9, 133.0 (C<sub>q</sub> phenyl), 135.4 (CH<sub>arom</sub>), 137.4, 137.6, 138.1, 138.5 (C<sub>q</sub> Bn); HRMS: C<sub>53</sub>H<sub>60</sub>O<sub>8</sub>Si + Na<sup>+</sup> requires 875.3950, found 875.3946.



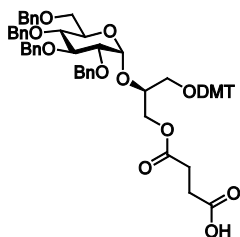
**2-O-(2,3,4,6-tetra-O-Benzyl-α-D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-sn-glycerol (18)**

To a cooled (0 °C) solution of alcohol **17** (1.85 g, 2.17 mmol) and Et<sub>3</sub>N (0.45 ml, 3.3 mmol) in DCM (11 ml) was added DMTr-Cl (881 mg, 2.60 mmol). The mixture was stirred for 2.5 hrs before MeOH (1.0 ml) was added. After stirring for an additional 15 minutes the reaction mixture was diluted with DCM (40 ml) and washed with a 1/1 mixture of sat. aq. NaHCO<sub>3</sub> and brine (30 ml). The aqueous layer was extracted with DCM (2 x 10 ml) and the combined organic layers were dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The residual oil was redissolved in THF (15ml) and, subsequently, TBAF (1M solution in THF, 7.8 ml) was added. The mixture was stirred for 3 hrs after which the volatiles were removed *in vacuo* and the residual oil was purified by silica gel column chromatography (EtOAc/PE/Et<sub>3</sub>N) yielding mono-alcohol **18** (1.77 g, 1.93 mmol, 89%) as a colourless oil. [α]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +40.6; IR: 737, 1030, 1065, 1250, 1508, 1609, 2927, 3487; <sup>1</sup>H NMR (400 MHz): δ = 2.85 (at, 1H, *J* = 5.0 Hz, CH<sub>2</sub>OH), 3.21 (dd, 1H, *J* = 6.1 Hz, 9.6 Hz, CHH glycerol), 3.35 (dd, 1H, *J* = 5.5 Hz, 9.6 Hz, CHH glycerol), 3.53 - 3.57 (m, 2H, H-2, H-4), 3.61 - 3.67 (m, 3H, H-6, H-6', CHH glycerol), 3.71 (s, 6H, 2 x OMe), 3.76 - 3.82 (m, 1H, CHH glycerol), 3.86 (m, 1H, CH glycerol), 3.96 - 4.04 (m, 2H, H-3, H-5), 4.46 (d, 1H, *J* = 10.8 Hz, CHH Bn), 4.47 (d, 1H, *J* = 12.4 Hz, CHH Bn), 4.58 (d, 1H, *J* = 11.6 Hz, CHH Bn), 4.63 (d, 1H, *J* = 12.0 Hz, CHH Bn), 4.80 (d, 1H, *J* = 10.4 Hz, CHH Bn), 4.82 (d, 1H, *J* = 10.4 Hz, CHH Bn), 4.96 (d, 1H, *J* = 10.8 Hz, CHH Bn), 4.99 (d, 1H, *J* = 3.6 Hz, H-1), 6.78 - 6.81 (m, 4H, H<sub>arom</sub>), 7.11 - 7.13 (m, 2H, H<sub>arom</sub>), 7.18 - 7.36 (m, 25H, H<sub>arom</sub>), 7.46 (d, 2H, *J* = 7.4 Hz, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 55.0 (2 x OMe), 63.4 (CH<sub>2</sub> glycerol), 63.9 (CH<sub>2</sub> glycerol), 68.5 (C-6), 70.5 (C-5), 72.6, 73.4, 75.0, 75.6 (4 x CH<sub>2</sub> Bn), 77.7 (C-4), 79.5 (C-2), 80.0 (CH glycerol), 81.8 (C-3), 86.3 (C<sub>q</sub> DMTr), 96.8 (C-1), 113.0 (CH<sub>arom</sub>), 126.7 - 129.0 (CH<sub>arom</sub>), 130.0 (CH<sub>arom</sub>), 135.8, 137.5, 137.9, 138.0, 138.6, 144.7, 158.4 (4 x C<sub>q</sub> Bn, 5 x C<sub>q</sub> DMTr); HRMS: C<sub>58</sub>H<sub>60</sub>O<sub>10</sub> + Na<sup>+</sup> requires 939.4079, found 939.4090.



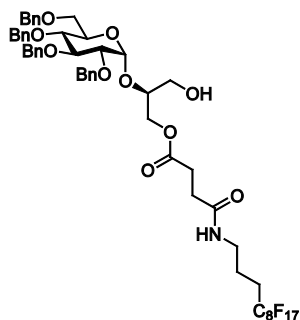
**1-O-([N,N-diisopropyl]-2-cyanoethyl-phosphoramidite)-2-O-(2,3,4,6-tetra-O-benzyl- $\alpha$ -D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-*sn*-glycerol (**19**)**

To a cooled (0 °C) solution of alcohol **18** (801 mg, 0.873 mmol) and Et<sub>3</sub>N (0.19 ml, 1.4 mmol) in DCM (6.0 ml) was added 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite (258 mg, 1.09 mmol). After stirring 30 min, the reaction was quenched by the addition of H<sub>2</sub>O (1.0 ml), diluted with DCM (20 ml) and washed with a 1/1 mixture of sat. aq. NaHCO<sub>3</sub> and brine (20 ml). The aqueous layer was extracted with DCM (2 x 10 ml) and the combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE/Et<sub>3</sub>N) gave phosphoramidite **19** (722 mg, 0.646 mmol, 74%) as a colourless oil. IR: 1030, 1250, 1508, 1605, 2928; <sup>31</sup>P NMR (161.7 MHz, CD<sub>3</sub>CN):  $\delta$  = 149.0, 149.4 (diastereoisomers); <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN, mixture of diastereoisomers):  $\delta$  = 1.04 - 1.12 (m, 12H, 4 x CH<sub>3</sub> isopropylamino), 2.40 - 2.42 (m, 2H, CH<sub>2</sub> cyanoethyl), 3.16 - 3.25 (m, 2H, CH<sub>2</sub> glycerol), 3.44 - 3.55 (m, 4H, H-2, H-4, 2 x CH isopropylamino), 3.60 - 3.88 (m, 13H, H-3, H-6, H-6', 2 x OMe, CH<sub>2</sub> glycerol, CH<sub>2</sub> cyanoethyl), 3.91 - 4.02 (m, 2H, H-5, CH glycerol), 4.48 - 4.61 (m, 5H, CH<sub>2</sub> Bn), 4.72 - 4.80 (m, 2H, C<sub>2</sub> Bn), 4.86 - 4.90 (m, 1H, CHH Bn), 5.15 - 5.18 (m, 1H, H-1), 6.80 (d, 4H, *J* = 8.9 Hz, H<sub>arom</sub>), 7.12 - 7.36 (m, 27H, H<sub>arom</sub>), 7.44 - 7.47 (m, 2H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 24.9 - 25.1 (4 x CH<sub>3</sub> isopropylamino), 43.7 - 43.8 (2 x CH isopropylamino), 55.8 (2 x OMe), 59.3 - 59.6 (CH<sub>2</sub> glycerol), 64.3 - 64.4 (CH<sub>2</sub> glycerol, CH<sub>2</sub> cyanoethyl), 69.9 (C-6), 71.4 - 71.5 (C-5), 72.8 - 72.9, 73.9, 75.4 - 75.5, 76.0 (4 x CH<sub>2</sub> Bn), 77.5 - 77.7 (CH glycerol), 78.8 (C-4), 81.0 (C-2), 82.5 (C-3), 87.2 (C<sub>q</sub> DMTr), 97.1 - 97.3 (C-1), 114.0 (CH<sub>arom</sub>), 127.7 - 129.3 (CH<sub>arom</sub>), 131.0 (CH<sub>arom</sub>), 136.9, 139.5, 139.5, 139.7, 139.8, 140.1, 146.1, 159.6 (4 x C<sub>q</sub> Bn, 5 x C<sub>q</sub> DMTr, C<sub>q</sub> cyanoethyl); HRMS: C<sub>67</sub>H<sub>77</sub>N<sub>2</sub>O<sub>11</sub>P + H<sup>+</sup> requires 1117.5338, found 1117.5337.



**2-O-(2,3,4,6-tetra-O-Benzyl- $\alpha$ -D-glucopyranosyl)-3-O-(4,4'-dimethoxytrityl)-1-O-succinyl-*sn*-glycerol (**20**)**

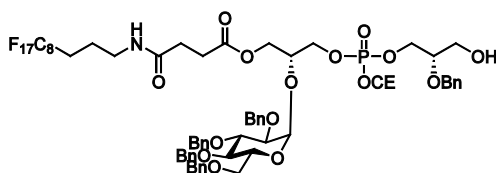
To a cooled (0 °C) solution of alcohol **18** (914 mg, 0.997 mmol) and Et<sub>3</sub>N (1.52 ml, 11.0 mmol) in DCM (10 ml) was added succinic anhydride (498 mg, 4.98 mmol). After stirring for 1 h the mixture was concentrated under reduced pressure, after which column chromatography (EtOAc/PE/Et<sub>3</sub>N) gave succinyl ester **20** (966 mg, 0.963 mmol, 96%) as a pale yellow oil. [ $\alpha$ ]<sub>D</sub><sup>20</sup> (CHCl<sub>3</sub>): +36.6; IR: 1030, 1153, 1246, 1508, 1609, 1713, 1736, 2928; <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN):  $\delta$  = 2.49 (s, 4H, 2 x CH<sub>2</sub> succinyl), 3.22 - 3.31 (m, 2H, CH<sub>2</sub> glycerol), 3.50 (dd, 1H, *J* = 3.5 Hz, 9.7 Hz, H-2), 3.57 (at, 1H, *J* = 9.5 Hz, H-4), 3.68 - 3.77 (m, 8H, H-6, H-6', 2 x OMe), 3.90 (at, 1H, *J* = 9.3 Hz, H-3), 3.93 - 3.98 (m, 1H, H-5), 4.01 - 4.08 (m, 1H, CH glycerol), 4.23 - 4.32 (m, 2H, CH<sub>2</sub> glycerol), 4.49 - 4.61 (m, 5H, CHH Bn, 2 x CH<sub>2</sub> Bn), 4.78 (d, 1H, *J* = 11.1 Hz, CHH Bn), 4.83 (d, 1H, *J* = 11.1 Hz, CHH Bn), 4.91 (d, 1H, *J* = 11.1 Hz, CHH Bn), 5.12 (d, 1H, *J* = 3.5 Hz, H-1), 6.84 (d, 4H, *J* = 8.9 Hz, H<sub>arom</sub>), 7.16 - 7.39 (m, 27H, H<sub>arom</sub>), 7.48 (d, 2H, *J* = 7.4 Hz, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>CN):  $\delta$  = 29.8, 30.3 (2 x CH<sub>2</sub> succinyl), 56.5 (2 x OMe), 64.1 (CH<sub>2</sub> glycerol), 65.9 (CH<sub>2</sub> glycerol), 70.5 (C-6), 72.2 (C-5), 73.7, 74.5, 76.1 (3 x CH<sub>2</sub> Bn), 76.2 (CH glycerol), 76.6 (CH<sub>2</sub> Bn), 79.4 (C-4), 81.5 (C-2), 82.9 (C-3), 87.9 (C<sub>q</sub> DMTr), 97.6 (C-1), 114.7 (CH<sub>arom</sub>), 128.4 - 129.9 (CH<sub>arom</sub>), 131.6 (CH<sub>arom</sub>), 137.3, 137.4, 139.9, 140.0, 140.2, 140.7, 146.6, 160.2 (5 x C<sub>q</sub> DMTr, 4 x C<sub>q</sub> Bn), 173.5, 174.9 (2 x C=O succinyl); HRMS: C<sub>62</sub>H<sub>64</sub>O<sub>13</sub> + Na<sup>+</sup> requires 1039.4239, found 1039.4237.



### 2-O-(2,3,4,6-tetra-O-Benzyl- $\alpha$ -D-glucopyranosyl)-1-O-(N-[3-perfluorooctylpropyl]-succinamidyl-*sn*-glycerol) (**21**)

To a solution of compound **20** (351 mg, 0.350 mmol), perfluorooctylpropylamine (119 mg, 0.250 mmol) and *N,N*-diisopropylethylamine (0.366 ml, 2.10 mmol) in a 2/1 mixture of DCM/DMF (5.0 ml) was added BOP (310 mg, 0.700 mmol). The mixture was stirred for 1.5 h before it was diluted with EtOAc (100 ml) and, subsequently, washed with sat. aq.  $\text{NaHCO}_3$  (2 x 50 ml),  $\text{H}_2\text{O}$  (2 x 50 ml) and brine (50 ml). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure, after which the residue was taken up in DCM (5.0 ml). After the addition of, respectively, triethylsilane (0.605 ml, 3.75

mmol) and dichloroacetic acid (0.308 ml, 3.75 mmol) the mixture was stirred 30 min and, subsequently, diluted with DCM (40 ml) and washed with a 1/1 mixture of sat. aq.  $\text{NaHCO}_3$  and brine (20 ml). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo*, after which the residue was partitioned between 80/20 acetonitrile/water and hexane and purified by FSPE as described in the general procedure. Fluorous compound **21** (263 mg, 0.224 mmol, 90%) was isolated as an amorphous solid.  $[\alpha]_{\text{D}}^{20}$  ( $\text{CHCl}_3$ ): +23.8; IR: 1026, 1065, 1146, 1200, 1547, 1644, 1736, 2924;  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.71 - 1.79 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.99 - 2.13 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 2.38 (t, 2H,  $J$  = 6.7 Hz,  $\text{CH}_2$  succinyl), 2.63 (t, 2H,  $J$  = 6.7 Hz,  $\text{CH}_2$  succinyl), 3.24 (dd, 2H,  $J$  = 6.8 Hz, 13.3 Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 3.54 - 3.75 (m, 6H, H-2, H-4, H-6, H-6',  $\text{CH}_2$  glycerol), 3.83 - 3.89 (m, 1H, CH glycerol), 3.91 - 3.96 (m, 1H, H-5), 4.00 (at, 1H,  $J$  = 9.4 Hz, H-3), 4.13 - 4.16 (m, 2H,  $\text{CH}_2$  glycerol), 4.46 - 4.50 (m, 2H, 2 x CHH Bn), 4.59 (d, 1H,  $J$  = 12.1 Hz, CHH Bn), 4.67 (d, 1H,  $J$  = 11.6 Hz, CHH Bn), 4.80 - 4.90 (m, 4H, H-1, 3 x CHH Bn), 4.95 (d, 1H,  $J$  = 11.0 Hz, CHH Bn), 5.85 (t, 1H,  $J$  = 5.9 Hz, NH), 7.12 - 7.15 (m, 2H,  $\text{H}_{\text{arom}}$ ), 7.24 - 7.37 (m, 18H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 20.8 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 28.3 (t,  $J$  = 22 Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 29.3, 30.7 (2 x  $\text{CH}_2$  succinyl), 38.5 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 61.8 ( $\text{CH}_2$  glycerol), 64.1 ( $\text{CH}_2$  glycerol), 68.4 (C-6), 70.9 (C-5), 73.5, 74.2, 75.1, 75.6 (4 x  $\text{CH}_2$  Bn), 77.7 (C-4), 78.8 (CH glycerol), 79.5 (C-2), 82.1 (C-3), 98.6 (C-1), 127.6 - 128.6 ( $\text{CH}_{\text{arom}}$ ), 137.4, 137.7, 138.0, 138.5 (4 x  $\text{C}_q$  Bn), 171.5, 172.6 (2 x C=O succinyl); HRMS:  $\text{C}_{52}\text{H}_{52}\text{F}_{17}\text{NO}_{10}$  +  $\text{Na}^+$  requires 1196.3212, found 1196.3210.

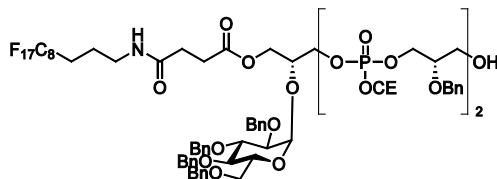


### Glucosyl glycerol phosphate dimer (**22**)

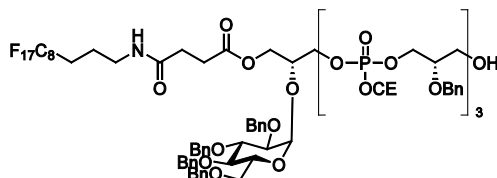
Monomer **21** (133 mg, 113  $\mu\text{mol}$ ) was coupled to glycerol phosphoramidite **6** (1.5 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Dimer **22** (153 mg, 104  $\mu\text{mol}$ , 92%) was obtained as a colorless oil.

$^{31}\text{P}$  NMR (161.7 MHz):  $\delta$  = -0.9, -0.9 (1P);  $^1\text{H}$  NMR (400 MHz):  $\delta$  = 1.70 - 1.78 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 1.99 - 2.12 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 2.26 - 2.67 (m, 7H, 2 x  $\text{CH}_2$  succinyl,  $\text{CH}_2$  cyanoethyl,  $\text{CH}_2\text{OH}$ ), 3.22 (dd, 2H,  $J$  = 6.7 Hz, 13.0 Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 3.54 - 3.60 (m, 1H, H-2), 3.61 - 3.73 (m, 6H, H-4, H-6, H-6', CH glycerol,  $\text{CH}_2$  glycerol), 3.84 - 3.95 (m, 2H, H-3, H-5), 4.01 - 4.31 (m, 9H, CH glycerol, 3 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$  cyanoethyl), 4.44 - 4.49 (m, 2H, 2 x CHH Bn), 4.56 - 4.73 (m, 5H, CHH Bn, 2 x  $\text{CH}_2$  Bn), 4.78 - 4.83 (m, 2H, 2 x CHH Bn), 4.92 - 4.96 (m, 1H, CHH Bn), 5.01 - 5.03 (m, 1H, H-1), 6.01 - 6.05 (m, 1H, NH), 7.12 - 7.14 (m, 2H,  $\text{H}_{\text{arom}}$ ), 7.25 - 7.37 (m, 23H,  $\text{H}_{\text{arom}}$ );  $^{13}\text{C}$  NMR (100 MHz):  $\delta$  = 19.3 - 19.4 ( $\text{CH}_2$  cyanoethyl), 20.7 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 28.3 (t,  $J$  = 22 Hz,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 29.4, 30.7 (2 x  $\text{CH}_2$  succinyl), 38.5 ( $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2\text{CH}_2\text{N}$ ), 60.5 (d,  $J$  = 5 Hz,  $\text{CH}_2$  glycerol), 62.0 (d,  $J$  = 5 Hz,  $\text{CH}_2$  cyanoethyl), 63.0 ( $\text{CH}_2$  glycerol), 66.1 - 66.6 (2 x  $\text{CH}_2$  glycerol), 68.3 (C-6), 70.9 (C-5), 72.0, 72.0 ( $\text{CH}_2$  Bn), 73.1, 73.2 ( $\text{CH}_2$  Bn), 73.5 ( $\text{CH}_2$  Bn), 73.9 - 74.1 (CH glycerol), 75.1, 75.5 (2 x  $\text{CH}_2$  Bn), 77.4 - 77.6 (C-4, CH glycerol), 79.6, 79.7 (C-2),

81.5 (C-3), 96.9, 97.1 (C-1), 116.4, 116.5 (C<sub>q</sub> cyanoethyl), 127.6 - 128.5 (CH<sub>arom</sub>), 137.6 - 137.7, 137.9, 138.6 (5 x C<sub>q</sub> Bn), 171.5, 172.3 (2 x C=O succinyl); HRMS: C<sub>65</sub>H<sub>68</sub>F<sub>17</sub>N<sub>2</sub>O<sub>15</sub>P + Na<sup>+</sup> requires 1493.3978, found 1493.3978.



(161.7 MHz):  $\delta$  = -1.4, -1.3, -1.3, -1.3 (1P), -1.0, -0.9 (1P); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 1.69 - 1.77 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.98 - 2.12 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.17 - 2.69 (m, 9H, 2 x CH<sub>2</sub> succinyl, 2 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>OH), 3.18 - 3.26 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.55 - 3.60 (m, 1H, H-2), 3.61 - 3.74 (m, 6H, H-4, H-6, H-6', CH glycerol, CH<sub>2</sub> glycerol), 3.76 - 3.82 (m, 1H, CH glycerol), 3.86 - 3.95 (m, 2H, H-3, H-5), 4.01 - 4.31 (m, 15H, CH glycerol, 5 x CH<sub>2</sub> glycerol, 2 x CH<sub>2</sub> cyanoethyl), 4.44 - 4.49 (m, 2H, 2 x CHH Bn), 4.56 - 4.65 (m, 5H, CHH Bn, 2 x CH<sub>2</sub> Bn), 4.69 - 4.72 (m, 2H, 2 x CHH Bn), 4.77 - 4.83 (m, 2H, 2 x CHH Bn), 4.91 - 4.95 (m, 1H, CHH Bn), 5.01 - 5.04 (m, 1H, H-1), 6.16 - 6.22 (m, 1H, NH), 7.12 - 7.15 (m, 2H, H<sub>arom</sub>), 7.25 - 7.37 (m, 28H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 19.2 - 19.4 (2 x CH<sub>2</sub> cyanoethyl), 20.6 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 28.2 (t, *J* = 22 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 29.3, 30.6 (2 x CH<sub>2</sub> succinyl), 38.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 60.4, 60.5 (CH<sub>2</sub> glycerol), 62.0 - 62.2 (2 x CH<sub>2</sub> cyanoethyl), 63.0 (CH<sub>2</sub> glycerol), 65.5 - 66.7 (4 x CH<sub>2</sub> glycerol), 68.3 (C-6), 70.8 (C-5), 72.0 (CH<sub>2</sub> Bn), 72.1, 72.2 (CH<sub>2</sub> Bn), 73.0, 73.1 (CH<sub>2</sub> Bn), 73.4 (CH<sub>2</sub> Bn), 73.8 - 74.1 (CH glycerol), 75.1 (CH<sub>2</sub> Bn), 75.2 - 75.4 (CH glycerol), 75.5 (CH<sub>2</sub> Bn), 77.4 - 77.5 (C-4, CH glycerol), 79.6 (C-2), 81.5 (C-3), 96.9, 97.0 (C-1), 116.5 - 116.6 (2 x C<sub>q</sub> cyanoethyl), 127.5 - 128.5 (CH<sub>arom</sub>), 137.1, 137.7 - 138.0, 138.5 (6 x C<sub>q</sub> Bn), 171.5, 172.3 (2 x C=O succinyl); HRMS: C<sub>78</sub>H<sub>84</sub>F<sub>17</sub>N<sub>3</sub>O<sub>20</sub>P<sub>2</sub> + Na<sup>+</sup> requires 1790.4744, found 1790.4744.

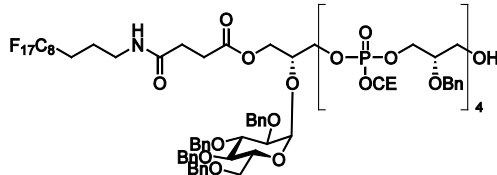


#### Glucosyl glycerol phosphate tetramer (24)

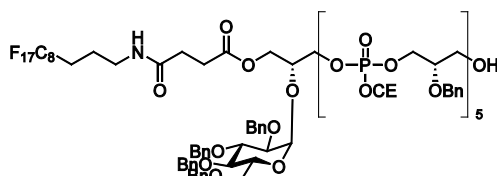
Trimer **23** (157 mg, 88.7  $\mu$ mol) was coupled to glycerol phosphoramidite **6** (1.5 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Tetramer **23** (169 mg, 82.0  $\mu$ mol, 92%) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz):  $\delta$  = -1.4 - -1.2 (2P), -0.9, -0.9, -0.9 (1P); <sup>1</sup>H NMR (400 MHz):  $\delta$  = 1.68 - 1.76 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.98 - 2.19 (m, 3H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N, CH<sub>2</sub>OH), 2.35 - 2.67 (m, 10H, 2 x CH<sub>2</sub> succinyl, 3 x CH<sub>2</sub> cyanoethyl), 3.18 - 3.25 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.54 - 3.60 (m, 1H, H-2), 3.61 - 3.75 (m, 6H, H-4, H-6, H-6', CH glycerol, CH<sub>2</sub> glycerol), 3.76 - 3.83 (m, 2H, 2 x CH glycerol), 3.85 - 3.95 (m, 2H, H-3, H-5), 4.01 - 4.30 (m, 21H, CH glycerol, 7 x CH<sub>2</sub> glycerol, 3 x CH<sub>2</sub> cyanoethyl), 4.44 - 4.49 (m, 2H, 2 x CHH Bn), 4.56 - 4.66 (m, 7H, CHH Bn, 3 x CH<sub>2</sub> Bn), 4.69 - 4.72 (m, 2H, 2 x CHH Bn), 4.76 - 4.83 (m, 2H, 2 x CHH Bn), 4.91 - 4.95 (m, 1H, CHH Bn), 5.01 - 5.04 (m, 1H, H-1), 6.12 - 6.19 (m, 1H, NH), 7.10 - 7.15 (m, 2H, H<sub>arom</sub>), 7.23 - 7.38 (m, 33H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz):  $\delta$  = 19.2 - 19.5 (3 x CH<sub>2</sub> cyanoethyl), 20.6 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 28.2 (t, *J* = 22 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 29.3, 30.6 (2 x CH<sub>2</sub> succinyl), 38.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 60.4, 60.5 (CH<sub>2</sub> glycerol), 62.0 - 62.2 (3 x CH<sub>2</sub> cyanoethyl), 63.1 (CH<sub>2</sub> glycerol), 65.5 - 66.6 (6 x CH<sub>2</sub> glycerol), 68.3 (C-6), 70.8 (C-5), 72.0 (CH<sub>2</sub> Bn), 72.1 - 72.2 (2 x CH<sub>2</sub> Bn), 73.0, 73.1 (CH<sub>2</sub> Bn), 73.4 (CH<sub>2</sub> Bn), 73.7 - 74.2 (CH glycerol), 75.1 (CH<sub>2</sub> Bn), 75.2 - 75.4 (2 x CH glycerol), 75.5 (CH<sub>2</sub> Bn), 77.4 - 77.5 (C-4, CH glycerol), 79.6 (C-2), 81.6 (C-3), 96.8, 97.0 (C-1), 116.6 - 116.7 (3 x C<sub>q</sub> cyanoethyl), 127.6 - 128.5 (CH<sub>arom</sub>), 137.2, 137.7 - 138.0,



138.5 (7 x C<sub>q</sub> Bn), 171.4, 172.3 (2 x C=O succinyl); HRMS: [C<sub>91</sub>H<sub>100</sub>F<sub>17</sub>N<sub>4</sub>O<sub>25</sub>P<sub>3</sub> + 2Na]<sup>2+</sup> requires 1055.2701, found 1055.2705.



74.3 μmol, 93%) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.4 - -1.1 (3P), -0.9, -0.9, -0.9 (1P); <sup>1</sup>H NMR (400 MHz): δ = 1.68 - 1.76 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.98 - 2.12 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.26 - 2.67 (m, 13H, 2 x CH<sub>2</sub> succinyl, 4 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>OH), 3.18 - 3.26 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.54 - 3.60 (m, 1H, H-2), 3.61 - 3.75 (m, 6H, H-4, H-6, H-6', CH glycerol, CH<sub>2</sub> glycerol), 3.76 - 3.83 (m, 3H, 3 x CH glycerol), 3.86 - 3.95 (m, 2H, H-3, H-5), 4.00 - 4.30 (m, 27H, CH glycerol, 9 x CH<sub>2</sub> glycerol, 4 x CH<sub>2</sub> cyanoethyl), 4.43 - 4.49 (m, 2H, 2 x CHH Bn), 4.56 - 4.67 (m, 9H, CHH Bn, 4 x CH<sub>2</sub> Bn), 4.69 - 4.71 (m, 2H, 2 x CHH Bn), 4.76 - 4.83 (m, 2H, 2 x CHH Bn), 4.91 - 4.95 (m, 1H, CHH Bn), 5.01 - 5.04 (m, 1H, H-1), 6.14 - 6.20 (m, 1H, NH), 7.10 - 7.15 (m, 2H, H<sub>arom</sub>), 7.23 - 7.39 (m, 38H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 19.3 - 19.4 (4 x CH<sub>2</sub> cyanoethyl), 20.6 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 28.2 (t, J = 23 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 29.3, 30.6 (2 x CH<sub>2</sub> succinyl), 38.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 60.4, 60.5 (CH<sub>2</sub> glycerol), 62.0 - 62.2 (4 x CH<sub>2</sub> cyanoethyl), 63.0 (CH<sub>2</sub> glycerol), 65.5 - 66.6 (8 x CH<sub>2</sub> glycerol), 68.3 (C-6), 70.8 (C-5), 71.9 (CH<sub>2</sub> Bn), 72.1 - 72.2 (3 x CH<sub>2</sub> Bn), 73.0, 73.1 (CH<sub>2</sub> Bn), 73.4 (CH<sub>2</sub> Bn), 73.7 - 74.0 (CH glycerol), 75.1 (CH<sub>2</sub> Bn), 75.1 - 75.4 (3 x CH glycerol), 75.5 (CH<sub>2</sub> Bn), 77.4 - 77.5 (C-4, CH glycerol), 79.6 (C-2), 81.5 (C-3), 96.8, 96.9 (C-1), 116.6 - 116.7 (4 x C<sub>q</sub> cyanoethyl), 127.5 - 128.4 (CH<sub>arom</sub>), 137.2, 137.7 - 138.0, 138.5 (8 x C<sub>q</sub> Bn), 171.4, 172.3 (2 x C=O succinyl); HRMS: [C<sub>104</sub>H<sub>116</sub>F<sub>17</sub>N<sub>5</sub>O<sub>30</sub>P<sub>4</sub> + 2Na]<sup>2+</sup> requires 1204.3101, found 1204.3100.



μmol, 90%) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.4 - -1.1 (4P), -0.9, -0.9, -0.9 (1P); <sup>1</sup>H NMR (400 MHz): δ = 1.68 - 1.76 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.98 - 2.12 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.31 - 2.67 (m, 15H, 2 x CH<sub>2</sub> succinyl, 5 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>OH), 3.17 - 3.25 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.54 - 3.59 (m, 1H, H-2), 3.61 - 3.74 (m, 6H, H-4, H-6, H-6', CH glycerol, CH<sub>2</sub> glycerol), 3.76 - 3.84 (m, 4H, 4 x CH glycerol), 3.85 - 3.94 (m, 2H, H-3, H-5), 4.00 - 4.31 (m, 33H, CH glycerol, 11 x CH<sub>2</sub> glycerol, 5 x CH<sub>2</sub> cyanoethyl), 4.44 - 4.48 (m, 2H, 2 x CHH Bn), 4.57 - 4.66 (m, 11H, CHH Bn, 5 x CH<sub>2</sub> Bn), 4.68 - 4.71 (m, 2H, 2 x CHH Bn), 4.77 - 4.82 (m, 2H, 2 x CHH Bn), 4.90 - 4.95 (m, 1H, CHH Bn), 5.01 - 5.04 (m, 1H, H-1), 6.17 - 6.23 (m, 1H, NH), 7.11 - 7.14 (m, 2H, H<sub>arom</sub>), 7.23 - 7.38 (m, 43H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 19.2 - 19.4 (5 x CH<sub>2</sub> cyanoethyl), 20.6 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 28.2 (t, J = 22 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 29.3, 30.5 (2 x CH<sub>2</sub> succinyl), 38.4 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 60.4, 60.5 (CH<sub>2</sub> glycerol), 62.0 - 62.2 (5 x CH<sub>2</sub> cyanoethyl), 63.1 (CH<sub>2</sub> glycerol), 65.5 - 66.6 (10 x CH<sub>2</sub> glycerol), 68.3 (C-6), 70.8 (C-5), 71.9 (CH<sub>2</sub> Bn), 72.1 - 72.2 (4 x CH<sub>2</sub> Bn), 73.0, 73.0 (CH<sub>2</sub> Bn), 73.4 (CH<sub>2</sub> Bn), 73.8 - 74.0 (CH glycerol), 75.1 (CH<sub>2</sub> Bn), 75.2 - 75.5 (4 x CH glycerol), 75.5 (CH<sub>2</sub> Bn), 77.4 - 77.6 (C-4, CH glycerol), 79.6 (C-2), 81.5 (C-3), 96.8, 96.9 (C-1), 116.6 - 116.8 (5 x C<sub>q</sub> cyanoethyl), 127.5 - 128.5 (CH<sub>arom</sub>), 137.2,

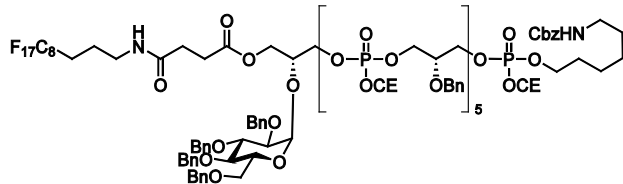
### Glucosyl glycerol phosphate pentamer (25)

Tetramer **24** (165 mg, 79.9 μmol) was coupled to glycerol phosphoramidite **6** (1.8 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Pentamer **25** (176 mg,

### Glucosyl glycerol phosphate hexamer (26)

Pentamer **25** (149 mg, 63.1 μmol) was coupled to glycerol phosphoramidite **6** (2.0 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Hexamer **26** (151 mg, 56.8

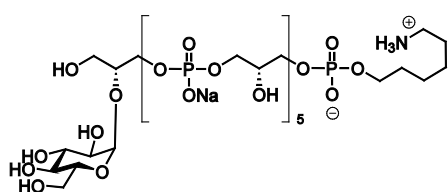
137.7 - 138.0, 138.5 (9 x C<sub>q</sub> Bn), 171.4, 172.3 (2 x C=O succinyl); HRMS: [C<sub>117</sub>H<sub>132</sub>F<sub>17</sub>N<sub>6</sub>O<sub>35</sub>P<sub>5</sub> + 2Na]<sup>2+</sup> requires 1352.8484, found 1352.8479.



### Glucosyl glycerol phosphate hexamer spacer (27)

Hexamer **26** (145 mg, 54.6 μmol) was coupled to spacer phosphoramidite **10** (2.5 eq), oxidized and purified (FSPE) using the general procedure as described above. Hexamer **27**

(147 mg, 48.7 μmol, 89%) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.4 - -1.1 (6P); <sup>1</sup>H NMR (400 MHz): δ = 1.25 - 1.40 (m, 4H, 2 x CH<sub>2</sub> hexylspacer), 1.44 - 1.52 (m, 2H, CH<sub>2</sub> hexylspacer), 1.60 - 1.68 (m, 2H, CH<sub>2</sub> hexylspacer), 1.68 - 1.76 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.98 - 2.12 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.35 - 2.67 (m, 16H, 2 x CH<sub>2</sub> succinyl, 6 x CH<sub>2</sub> cyanoethyl), 3.12 - 3.25 (m, 4H, CH<sub>2</sub>-N hexylspacer, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.54 - 3.59 (m, 1H, H-2'), 3.61 - 3.68 (m, 2H, H-4, H-6), 3.70 - 3.75 (m, 1H, H-6'), 3.76 - 3.84 (m, 5H, 5 x CH glycerol), 3.85 - 3.94 (m, 2H, H-3, H-5), 4.00 - 4.31 (m, 39H, CH glycerol, 12 x CH<sub>2</sub> glycerol, 6 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>-O hexylspacer), 4.44 - 4.48 (m, 2H, 2 x CHH Bn), 4.57 - 4.65 (m, 11H, CHH Bn, 5 x CH<sub>2</sub> Bn), 4.68 - 4.71 (m, 2H, 2 x CHH Bn), 4.76 - 4.83 (m, 2H, 2 x CHH Bn), 4.90 - 4.95 (m, 1H, CHH Bn), 5.01 - 5.13 (m, 4H, H-1, CH<sub>2</sub> Cbz, NH Cbz), 6.11 - 6.18 (m, 1H, NH), 7.11 - 7.14 (m, 2H, H<sub>arom</sub>), 7.22 - 7.39 (m, 48H, H<sub>arom</sub>); <sup>13</sup>C NMR (100 MHz): δ = 19.2 - 19.5 (6 x CH<sub>2</sub> cyanoethyl), 20.6 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 24.8 (CH<sub>2</sub> hexylspacer), 25.9 (CH<sub>2</sub> hexylspacer), 28.2 (t, *J* = 22 Hz, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 29.3 (CH<sub>2</sub> succinyl), 29.6 (CH<sub>2</sub> hexylspacer), 29.9 (d, *J* = 7 Hz, CH<sub>2</sub> hexylspacer), 30.5 (CH<sub>2</sub> succinyl), 38.3 (F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 40.7 (CH<sub>2</sub>-N hexylspacer), 61.8 - 62.1 (6 x CH<sub>2</sub> cyanoethyl), 63.0 (CH<sub>2</sub> glycerol), 65.5 - 66.4 (11 x CH<sub>2</sub> glycerol, CH<sub>2</sub> Cbz), 68.3 - 68.4 (C-6, CH<sub>2</sub>-O hexylspacer), 70.8 (C-5), 72.1 - 72.2 (6 x CH<sub>2</sub> Bn), 72.9, 73.0 (CH<sub>2</sub> Bn), 73.4 (CH<sub>2</sub> Bn), 73.7 - 74.0 (CH glycerol), 75.0 (CH<sub>2</sub> Bn), 75.3 - 75.5 (5 x CH glycerol), 77.4 (C-4), 79.6 (C-2), 81.5 (C-3), 96.8, 96.9 (C-1), 116.5 - 116.7 (6 x C<sub>q</sub> cyanoethyl), 127.5 - 128.5 (CH<sub>arom</sub>), 136.6, 137.2, 137.7 - 138.0, 138.5 (9 x C<sub>q</sub> Bn, C<sub>q</sub> Cbz), 156.3 (C=O Cbz), 171.3, 172.2 (2 x C=O succinyl); HRMS: [C<sub>134</sub>H<sub>155</sub>F<sub>17</sub>N<sub>8</sub>O<sub>40</sub>P<sub>6</sub> + 2Na]<sup>2+</sup> requires 1535.9156, found 1535.9153.

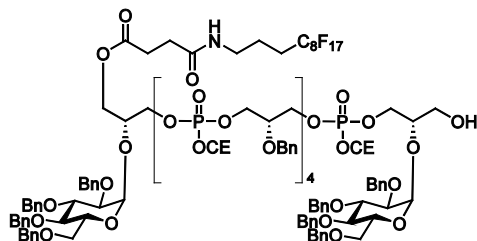


### Glucosyl glycerol phosphate hexamer (27)

Protected hexamer **27** (139 mg, 46.3 μmol) was treated with aqueous ammonia as described above. The intermediate hexamer (98.5 mg, 44.1 μmol, 95%) was obtained as an amorphous white solid. Analytical data intermediate: <sup>31</sup>P NMR (161.7 MHz, D<sub>2</sub>O): δ = 0.9 - 1.1 (5P), 1.2 (1P); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ = 0.80 - 1.10 (m, 6H, 3 x

CH<sub>2</sub> hexylspacer), 1.21 - 1.36 (m, 2H, CH<sub>2</sub> hexylspacer), 2.59 - 2.78 (m, 2H, CH<sub>2</sub>-N hexylspacer), 3.24 - 4.05 (m, 38H, H-2, H-3, H-4, H-5, H-6, H-6', 12 x CH<sub>2</sub> glycerol, 6 x CH glycerol, CH<sub>2</sub>-O hexylspacer), 4.16 - 5.02 (m, 22H, H-1, 9 x CH<sub>2</sub> Bn, CH<sub>2</sub> Cbz, NH Cbz), 6.66 - 7.14 (m, 50H, H<sub>arom</sub>); HRMS: [C<sub>101</sub>H<sub>127</sub>N<sub>3</sub>O<sub>38</sub>P<sub>6</sub> + 2NH<sub>4</sub>]<sup>2+</sup> requires 1092.3586, found 1092.3590. A portion of the intermediate (34.5 mg, 15.4 μmol) was deprotected with Pd (0)/H<sub>2</sub> using the standard procedure. Glucosylated hexamer **2** (19.7 mg, 15.0 μmol, 97%) was obtained as an amorphous white solid. <sup>31</sup>P NMR (161.7 MHz, D<sub>2</sub>O): δ = 1.2 (1P), 1.2 - 1.3 (4P), 1.3 (1P); <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O): δ = 1.39 - 1.44 (m, 4H, 2 x CH<sub>2</sub> hexylspacer), 1.61 - 1.70 (m, 4H, 2 x CH<sub>2</sub> hexylspacer), 2.99 (t, 2H, *J* = 7.5 Hz, CH<sub>2</sub>-N hexylspacer), 3.39 (at, 1H, *J* = 9.6 Hz, H-4), 3.52 (dd, 1H, *J* = 3.9 Hz, 9.9 Hz, H-2), 3.71 - 3.76 (m, 4H, H-3, H-6, CH<sub>2</sub> glycerol), 3.80 - 4.05 (m, 32H, H-5, H-6', 6 x CH

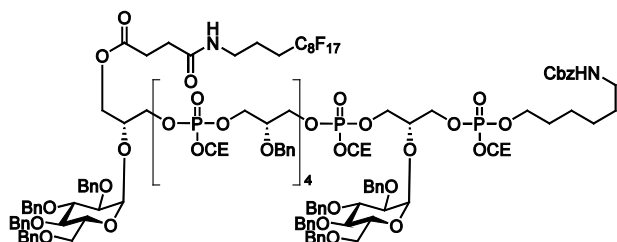
glycerol, 11 x CH<sub>2</sub> glycerol, CH<sub>2</sub>-O hexylspacer), 4.14 - 4.17 (m, 1H, CH glycerol), 5.15 (d, 1H, *J* = 3.8 Hz, H-1); <sup>13</sup>C NMR (150 MHz, D<sub>2</sub>O): δ = 25.3, 26.0, 27.5, 30.3 (4 x CH<sub>2</sub> hexylspacer), 40.3 (CH<sub>2</sub>-N hexylspacer), 61.4 (C-6), 62.2 (CH<sub>2</sub> glycerol), 65.2 (d, *J* = 6 Hz, CH<sub>2</sub> glycerol), 66.9 - 67.1 (CH<sub>2</sub>-O hexylspacer, 11 x CH<sub>2</sub> glycerol), 70.3 - 70.5 (5 x CH glycerol, C-4), 72.4 (C-2), 72.9 (C-5), 73.8 (C-3), 77.7 (d, *J* = 8 Hz, CH glycerol), 98.7 (C-1); HRMS: C<sub>30</sub>H<sub>67</sub>NO<sub>36</sub>P<sub>6</sub> + H<sup>+</sup> requires 1204.1941, found 1204.1948.



#### **bis-Glucosyl glycerol phosphate hexamer (28)**

Pentamer **25** (22.5 mg, 9.52 μmol) was coupled to glucosyl-glycerol phosphoramidite **19** (3.0 eq), oxidized, detritylated and purified (FSPE) using the general procedure as described above. Bis-glucosylated hexamer **28** (25.7 mg, 8.31 μmol, 87%) was obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.4 - -1.3 (2P), -1.2, -1.0 (3P); <sup>1</sup>H NMR (400 MHz): δ

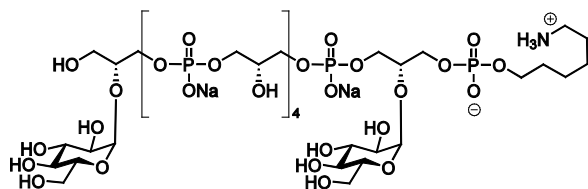
= 1.66 - 1.82 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.97 - 2.12 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.28 - 2.68 (m, 15H, 2 x CH<sub>2</sub> succinyl, 5 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>OH), 3.17 - 3.28 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.51 - 3.98 (m, 19H, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 2 x H-6, 2 x H-6', 5 x CH glycerol, CH<sub>2</sub> glycerol), 4.00 - 4.31 (m, 33H, CH glycerol, 11 x CH<sub>2</sub> glycerol, 5 x CH<sub>2</sub> cyanoethyl), 4.42 - 4.48 (m, 4H, 4 x CHH Bn), 4.56 - 4.64 (m, 10H, 2 x CHH Bn, 4 x CH<sub>2</sub> Bn), 4.67 - 4.71 (m, 3H, 3 x CHH Bn), 4.76 - 4.84 (m, 5H, 5 x CHH Bn), 4.90 - 4.95 (m, 3H, H-1, 2 x CHH Bn), 5.01 - 5.03 (m, 1H, H-1), 6.02 - 6.09 (m, 1H, NH), 7.09 - 7.16 (m, 4H, H<sub>arom</sub>), 7.22 - 7.38 (m, 56H, H<sub>arom</sub>); HRMS: [C<sub>144</sub>H<sub>160</sub>F<sub>17</sub>N<sub>6</sub>O<sub>40</sub>P<sub>5</sub> + 2Na]<sup>2+</sup> requires 1568.9452, found 1568.9454.



#### **bis-Glucosylglycerol phosphate hexamer spacer (29)**

Hexamer **28** (24.5 mg, 7.92 μmol) was coupled to spacer phosphoramidite **10** (5 eq), oxidized and purified (FSPE) using the general procedure as described above. Hexamer **29** (24.3 mg, 7.06 μmol, 89%) was

obtained as a colorless oil. <sup>31</sup>P NMR (161.7 MHz): δ = -1.4 - -1.0 (6P); <sup>1</sup>H NMR (400 MHz): δ = 1.26 - 1.37 (m, 4H, 2 x CH<sub>2</sub> hexylspacer), 1.41 - 1.49 (m, 2H, CH<sub>2</sub> hexylspacer), 1.59 - 1.68 (m, 2H, CH<sub>2</sub> hexylspacer), 1.68 - 1.77 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 1.97 - 2.11 (m, 2H, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 2.30 - 2.66 (m, 16H, 2 x CH<sub>2</sub> succinyl, 6 x CH<sub>2</sub> cyanoethyl), 3.10 - 3.25 (m, 4H, CH<sub>2</sub>-N hexylspacer, F<sub>17</sub>C<sub>8</sub>CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>N), 3.54 - 3.94 (m, 18H, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 2 x H-6, 2 x H-6', 4 x CH glycerol), 4.00 - 4.30 (m, 40H, 2 x CH glycerol, 12 x CH<sub>2</sub> glycerol, 6 x CH<sub>2</sub> cyanoethyl, CH<sub>2</sub>-O hexylspacer), 4.40 - 4.48 (m, 4H, 4 x CHH Bn), 4.55 - 4.64 (m, 10H, 2 x CHH Bn, 4 x CH<sub>2</sub> Bn), 4.68 - 4.71 (m, 4H, 4 x CHH Bn), 4.76 - 4.83 (m, 4H, 4 x CHH Bn), 4.90 - 4.95 (m, 2H, 2 x CHH Bn), 5.01 - 5.13 (m, 5H, 2 x H-1, CH<sub>2</sub> Cbz, NH Cbz), 6.04 - 6.11 (m, 1H, NH), 7.08 - 7.15 (m, 4H, H<sub>arom</sub>), 7.22 - 7.38 (m, 61H, H<sub>arom</sub>); HRMS: [C<sub>161</sub>H<sub>183</sub>F<sub>17</sub>N<sub>8</sub>O<sub>45</sub>P<sub>6</sub> + 2H]<sup>2+</sup> requires 1730.0305, found 1730.0312.



**bis-Glucosyl glycerol phosphate hexamer (4)**

Protected hexamer **29** (22.5 mg, 6.54  $\mu\text{mol}$ ) was treated with aqueous ammonia as described above. The intermediate hexamer (17.1 mg, 6.42  $\mu\text{mol}$ , 98%) was obtained as an amorphous white

solid. Analytical data intermediate:  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 0.9 - 1.2 (6P);  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 0.76 - 1.10 (m, 6H, 3 x  $\text{CH}_2$  hexylspacer), 1.21 - 1.34 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.55 - 2.76 (m, 2H,  $\text{CH}_2\text{-N}$  hexylspacer), 3.11 - 4.14 (m, 44H, 2 x H-2, 2 x H-3, 2 x H-4, 2 x H-5, 2 x H-6, 2 x H-6', 12 x  $\text{CH}_2$  glycerol, 6 x CH glycerol,  $\text{CH}_2\text{-O}$  hexylspacer), 4.15 - 4.84 (m, 28H, 2 x H-1, 12 x  $\text{CH}_2$  Bn,  $\text{CH}_2$  Cbz), 4.96 - 5.04 (m, 1H, NH Cbz), 6.61 - 7.17 (m, 65H,  $\text{H}_{\text{arom}}$ ); HRMS:  $[\text{C}_{128}\text{H}_{155}\text{NO}_{43}\text{P}_6 + 2\text{NH}_4]^{2+}$  requires 1308.4554, found 1308.4563. A portion of the intermediate (15.6 mg, 5.85  $\mu\text{mol}$ ) was deprotected with Pd (0)/ $\text{H}_2$  using the standard procedure. Bis-glucosylated hexamer **4** (8.43 mg, 5.71  $\mu\text{mol}$ , 98%) was obtained as an amorphous white solid.  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 0.9 (1P), 1.2 - 1.3 (5P);  $^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 1.40 - 1.44 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.62 - 1.70 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.99 (t, 2H,  $J$  = 7.5 Hz,  $\text{CH}_2\text{-N}$  hexylspacer), 3.36 - 3.41 (m, 2H, 2 x H-4), 3.48 - 3.53 (m, 2H, 2 x H-2), 3.71 - 3.77 (m, 6H, 2 x H-3, 2 x H-6,  $\text{CH}_2$  glycerol), 3.80 - 4.05 (m, 33H, 2 x H-5, 2 x H-6', 5 x CH glycerol, 11 x  $\text{CH}_2$  glycerol,  $\text{CH}_2\text{-O}$  hexylspacer), 4.07 - 4.10 (m, 1H, CH glycerol), 5.15 (d, 2H,  $J$  = 3.4 Hz, 2 x H-1);  $^{13}\text{C}$  NMR (150 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  = 25.5, 26.1, 27.6, 30.5 (4 x  $\text{CH}_2$  hexylspacer), 40.4 ( $\text{CH}_2\text{-N}$  hexylspacer), 61.2 (2 x C-6), 61.7 ( $\text{CH}_2$  glycerol), 65.3 (d,  $J$  = 5 Hz,  $\text{CH}_2$  glycerol), 66.1 (d,  $J$  = 5 Hz,  $\text{CH}_2$  glycerol), 67.1 - 67.3 ( $\text{CH}_2\text{-O}$  hexylspacer, 10 x  $\text{CH}_2$  glycerol), 70.4 - 70.7 (4 x CH glycerol, 2 x C-4), 72.5, 72.5 (2 x C-2), 72.9, 73.0 (2 x C-5), 73.9, 74.0 (2 x C-3), 76.4 (t,  $J$  = 8 Hz, CH glycerol), 77.8 (d,  $J$  = 8 Hz, CH glycerol), 98.7, 98.8 (2 x C-1); HRMS:  $\text{C}_{36}\text{H}_{77}\text{NO}_{41}\text{P}_6 + \text{H}^+$  requires 1366.2469, found 1366.2474.

## References

1. Hogendorf, W.F.J.; Meeuwenoord, N.; Overkleeft, H.S.; Filippov, D.V.; Laverde, D.; Kropec, A.; Huebner, J.; van der Marel, G.A.; Codée, J.D.C. *Chem. Commun.* **2011**, *47*, 8961-8963.
2. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Fabretti, F.; Sange, T.; Holst, O.; Huebner, J. *Infect. Immun.* **2006**, *74*, 5703-5712.
3. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Sava, I.; Ye, L.; Bychowska, A.; Holst, O.; Huebner, J. *Plos One* **2011**, *6*, e17839.
4. Theilacker, C.; Kropec, A.; Hammer, F.; Sava, I.; Wobser, D.; Sakinc, T.; Codée, J.D.C.; Hogendorf, W.F.J.; Van der Marel, G.A.; Huebner, J. *J. Infect. Dis.* **2012**, *205*, 1076-1085.
5. Gladysz, J.A.; Curran, D.P.; Horváth, I.T., *Handbook of Fluorous Chemistry*; Eds: Wiley-VCH: Weinheim, **2004**.
6. Curran, D.P. *Aldrichimica Acta* **2006**, *39*, 3-9.
7. Zhang, W.; Curran, D.P. *Tetrahedron* **2006**, *62*, 11837-11865.
8. Zhang, W.; Cai, C. *Chem. Commun.* **2008**, 5686-5694.
9. Zhang, W. *Chem. Rev.* **2009**, *109*, 749-795.
10. Zhang, W. *Green. Chem.* **2009**, *11*, 911-920.
11. Hogendorf, W.F.J.; Lameijer, L.N.; Beenakker, T.J.M.; Overkleeft, H.S.; Filippov, D.V.; Codée, J.D.C.; Van der Marel, G.A. *Org. Lett.* **2012**, *14*, 848-851.
12. Hogendorf, W.F.J.; Kropec, A.; Filippov, D.V.; Overkleeft, H.S.; Huebner, J.; Van der Marel, G.A. *Carbohydr. Res.* **2012**, *356*, 142-151.
13. Ali, A.; Van den Berg, R.J.B.H.N.; Overkleeft, H.S.; Filippov, D.V.; Van der Marel, G.A.; Codée, J.D.C. *Tetrahedron Lett.* **2009**, *50*, 2185-2188.
14. Hogendorf, W.F.J.; Van den Bos, L.J.; Overkleeft, H.S.; Codée, J.D.C.; van der Marel, G.A. *Bioorg. Med. Chem.* **2010**, *18*, 3668-3678.
15. Zheng, W.; DeMattei, J.A.; Wu, J.-P.; Duan, J.J.-W.; Cook, L.R.; Oinuma, H.; Kishi, Y. *J. Am. Chem. Soc.* **1996**, *118*, 7946-7968.
16. Beaucage, S.L.; Iyer, R.P. *Tetrahedron* **1992**, *48*, 2223-2311.
17. Reese, C.B. *Org. Biomol. Chem.* **2005**, *3*, 3851-3868.
18. Schmidt, R.R. *Angew. Chem.* **1986**, *98*, 213-236; *Angew. Chem. Int. Ed.* **1986**, *25*, 215-235.
19. Eby, R.; Schuerch, C. *Carbohydr. Res.* **1974**, *34*, 79-90.
20. Walvoort, M.T.C.; Lodder, G.; Overkleeft, H.S.; Codée, J.D.C.; Van der Marel, G.A. *J. Org. Chem.* **2010**, *75*, 7990-8002.
21. Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2002**, *43*, 5573-5577.
22. Adinolfi, M.; Iadonisi, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 6479-6482.

## Chapter 6

---

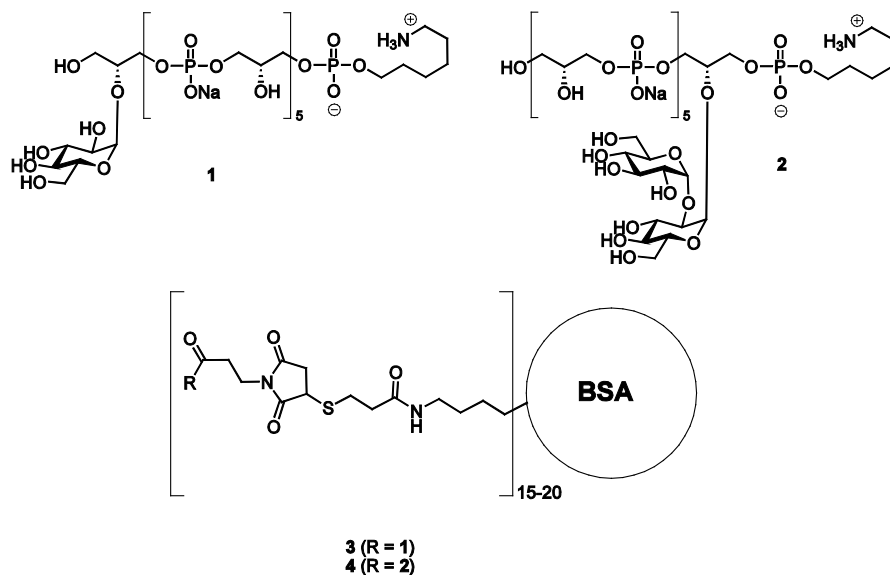
# Synthesis of TA-Protein Conjugates and Their Immunological Evaluation

### Introduction

Many Gram positive bacteria, such as *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pneumonia* and *Clostridium difficile*, pose an emerging risk in healthcare due to the increasing resistance against antibiotics.<sup>1</sup> *E. faecalis* has become second in line of most common nosocomial infections and this normally harmless commensal poses a considerable threat to immunocompromized and critically ill patients as the source of infections such as bacteremia, peritonitis, endocarditis, urinary tract and wound infections.<sup>2</sup> As a result there is a growing interest in the development of alternative treatments and preventive strategies to combat these bacteria. With the identification of several virulence factors of *E. faecalis*, the possibility for the development of prophylactic and therapeutic vaccine strategies becomes an attractive option.<sup>3</sup>

Several virulence factors involved in the pathogenesis of enterococcal infections have been identified, including cell surface exposed proteins, involved in the adherence of the bacteria to extracellular structures, biofilm formation and colonization events, and cell-wall carbohydrate antigens, such as capsular polysaccharides (CPs) and lipoteichoic acids (LTAs).<sup>4</sup> In 2006, Theilacker *et al.* demonstrated that non-encapsulated strains of *E. faecalis*, which account for about half of the clinical isolates, are opsonized by rabbit antibodies raised against purified LTA derived from these bacteria.<sup>5</sup> Passive immunization of infected mice with this antiserum led to clearance of *E. faecalis* and *S. epidermis* in the bloodstream. Additionally, it was revealed that the rabbit antiserum raised against *E. faecalis* LTA opsonized a selection of clinically relevant enterococcal, streptococcal and staphylococcal strains in an opsonophagocytic killing assay (OPA). These results indicate that the opsonic antibodies were raised against the common non-substituted 1,3-glycerol phosphate LTA-backbone. With the aid of synthetic TA oligomers, described in **chapter 3** and **chapter 5** of this thesis, it was shown that relatively short non-substituted oligoglycerol phosphates were capable of inhibiting killing by opsonic antibodies raised against native *E. faecalis* LTA.<sup>6</sup> Using the same opsonophagocytic killing inhibition assay (OPIA) it was revealed that the incorporation of a single  $\alpha$ -

glucosyl substituent in a TA hexamer fragments led to a significantly improved inhibitor, indicating that the glucosylated oligomers could function as potent antigens.<sup>7</sup> Notably, this type of substitution does not occur in *E. faecalis* LTA. The identification of TA hexamer **1** (Figure 1) as a possible synthetic antigen invites the use of this compound in a model vaccine for passive and/or active immunization strategies. This chapter describes the development of a semi-synthetic TA-carrier protein conjugate as a possible vaccine candidate.



**Figure 1.** Active **1** and inactive **2** and their conjugates with BSA (**3,4**).

## Results and Discussion

Small (carbohydrate) antigens, such as fragment **1**, are generally poorly immunogenic and only induce a T-cell independent immune response. A well-established approach to improve this low immune response comprises the conjugation of the antigen to an immunogenic carrier protein. This leads to T-cell dependent immune response, invoking immunological memory, affinity maturation, and IgM to IgG isotype switching.<sup>8-10</sup> Conjugate vaccines<sup>11-17</sup> are capable of eliciting an immune response in infants below the age of two years and this type of vaccines is now commonly used in broad national vaccination programs worldwide. A notable example of a semi-synthetic glycoconjugate vaccine that is currently used in Cuba and Vietnam and other countries is represented by Quimihib®, a vaccine developed against *Haemophilus influenzae* Type *b* by Verez-Bencomo *et al.* comprising a fully synthetic antigen covalently linked to a tetanus toxoid (TTd) carrier protein.<sup>17</sup> Various (semi)-synthetic conjugate vaccines against important pathogens and specific cancer types are currently under development.<sup>8,9,18</sup>

For the development of conjugate vaccines several proteins can be used as immunogenic carrier, such as the tetanus toxoid mentioned above, diphtheria toxoid (DTd), CRM197 (a non-toxic recombinant form of DTd), keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Because of its stability, ease of handling and modification possibilities, the latter protein was selected as a carrier in the first experimental vaccine. Its favorable molecular weight (~67 kD) as well as its non-glycosylated nature allows the use of several analytical techniques to characterize the conjugate.<sup>10</sup>

Two synthetic antigens were selected for conjugation to BSA: TA hexamer **1**, featuring a glycosyl substituent on the terminal glycerol phosphate moiety and kojibiosyl-containing fragment **2** (Figure 1). The former was selected because it represent the most active synthetic fragment from the assembled TA-oligomer library, as indicated by the OPIA described in **chapter 3**. The latter structure was used, because this structure, although it includes the natural glycosyl substituent found in *E. faecalis* LTA, showed a relatively poor inhibitory activity and therefore the projected conjugate serves as a relevant control compound. The conjugation of haptens to carrier proteins such as BSA can be accomplished with the aid of various chemical strategies and the aminohexyl functionality present on TA fragments **1** and **2** allows the installment of a conjugation handle of choice. It was decided to adapt the conjugation strategy used by Verez-Bencomo *et al.* in the development of the Quimihib®-vaccine, because of the structural similarities between the Hib capsular polysaccharide and the TA-oligomers. In this procedure the phosphodiester glycan fragments are equipped with a maleimide functionality and the lysine side chain amino functions in the protein are decorated with thiopropionyl moieties to allow for a robust and reliable conjugation reaction through a Michael type addition.<sup>17,19</sup>

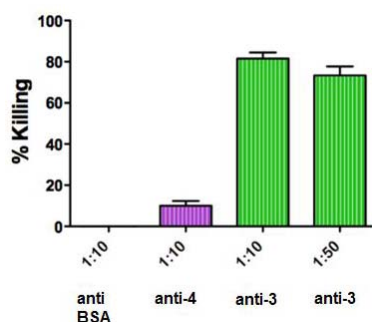
As depicted in Scheme 1, treatment of **1** and **2** with the hydroxysuccinimide ester of 3-maleimidopropionic acid (**5**) in a basic mixture of DMF and H<sub>2</sub>O gave, after size exclusion chromatography, the maleimides **6** and **7** in 67 and 86% yield, respectively. BSA (**8**) was treated with the homodisulfide of 3-thiopropionic acid hydroxysuccinimide ester (**9**) in a mixture of DMF and phosphate buffer saline (PBS, pH 8.0). After reduction of the disulfide bonds with dithiothreitol (DTT) and dialysis under inert atmosphere, the thiolated BSA (**10**) was obtained.<sup>19</sup> Quantification of the thiols present on the protein was performed by reaction of **10** with Ellman's reagent followed by UV<sub>412</sub> analysis, indicating the presence of an additional ~26 thiol functionalities (total amount of thiol groups ~43) compared to DTT treated BSA (**8**, ~17 thiols per protein). Maleimides **6** and **7** were conjugated with thiolated-BSA **10** (1.2 eq of maleimide with respect to the total amount of thiol groups were used) to give conjugates **3** and **4**, which were purified by dialysis and analyzed on protein and sugar content. Based on UV<sub>280</sub> spectrometry and phenol/H<sub>2</sub>SO<sub>4</sub> colorimetric assays a sugar:protein ratio of ~1.5:1 was established for both conjugates. SDS-PAGE analysis of the conjugates showed broad bands around ~95 kDa and ~100 kDa, respectively,



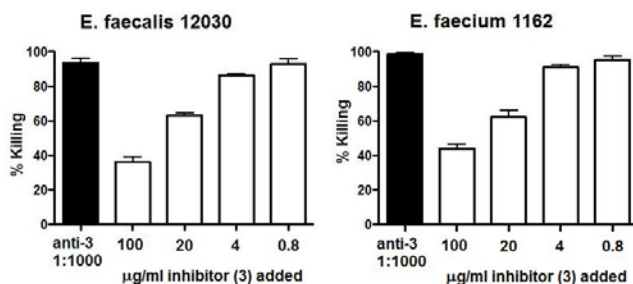


indicating protein:sugar ratio's of ~2.4:1 and 2.0:1 for 3 and 4, respectively. These data suggest a substitution rate of around 20 (+/- 5) TA-fragments per BSA (see Figure 2).

To evaluate the immunological activity, rabbits were repeatedly immunized s.c. and subsequently i.v. with conjugate 3, 4 or native BSA. After five weeks, serum was obtained and tested regarding the presence of opsonic antibodies against *E. faecalis* in an opsonophagocytic killing assay (OPA). In this assay appropriate serum dilutions (100  $\mu$ l) were added to a mixture of human leukocytes (100  $\mu$ l,  $2 \times 10^7$  cells/ml), complement proteins (preadsorbed baby rabbit serum, 100  $\mu$ l) and *E. faecalis* (100  $\mu$ l,



**Figure 3.** Opsonic killing of *E. faecalis* by rabbit antiserum raised to conjugates 3 and 4. Anti-BSA is used as a control (left).



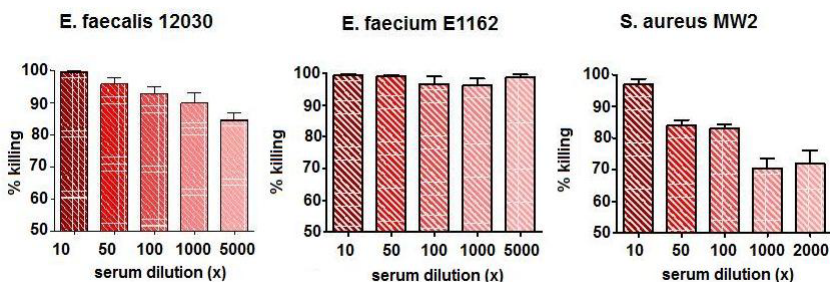
**Figure 4.** OPIA assay of different amounts of construct 3 using anti-3 serum against *E. faecium* and *E. faecalis*. The black bars on the left represent the killing of the bacterium by anti-3 in the OPA when no inhibitor is present (negative control).

2  $\times 10^7$  CFU/ml). After incubating 90 minutes at 37  $^{\circ}$ C on a rotor rack, the remaining number of viable enterococcal colony forming units (CFUs) was quantified. The results of this OPA are depicted in Figure 3, where it can be seen that only the serum raised against conjugate 3 was able to significantly kill *E. faecalis*. Anti-4 serum gave rise to a low amount of opsonic antibodies and the anti-BSA serum showed no opsonic killing at all. These results show that fragment 1 not only effectively binds to opsonic antibodies raised against native enterococcal LTA, as revealed in chapter 3, but that it can also be used as an immunogen when present as a BSA conjugate *in vivo*. As a control experiment an opsonophagocytic inhibition assay (OPIA) was performed with anti-3 serum on *E.*

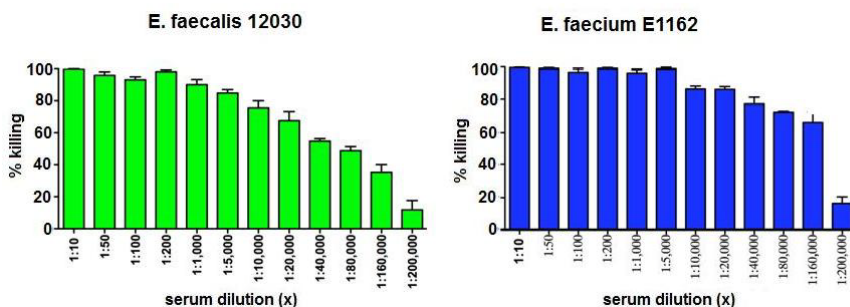
*faecalis* and *E. faecium* using conjugate 3 as an inhibitor. Figure 4 demonstrates the specificity of the opsonic killing observed. While serum dilutions of 1:1,000 were effectively able to kill *E. faecalis* and *E. faecium*, this killing activity could be removed by absorption with conjugate 3 in a dose-dependent fashion.

As described above, Theilacker *et al.* observed that serum raised against native enterococcal LTA showed cross-reactivity towards a selection of Gram-positive species.<sup>6</sup> Therefore, it was explored whether the anti-3 rabbit serum was able to

opsonize some of these bacteria. To this end, the previously mentioned OPA was performed using strains of *Enterococcus faecalis*, *Enterococcus faecium* and *Staphylococcus aureus*. As revealed in Figure 5, *E. faecalis* is readily opsonized by the serum. The closely related *E. faecium* was effectively killed in the OPA and also *S. aureus* was opsonized by anti-3 serum to a significant extent (i.e. about 70% killing at a serum dilution of 1:1,000). Because the OPA showed that the serum raised against conjugate 3 could be diluted 5000-fold with minimal loss of activity against *E. faecalis* and *E. faecium* the serum was investigated at higher dilutions (Figure 6). This revealed that *E. faecalis* is significantly opsonized by this serum up to a 20.000-fold dilution (~70 % killing). Notably, the serum proved even more potent against *E. faecium* and could be diluted 160.000 fold before a significant drop in activity was observed.



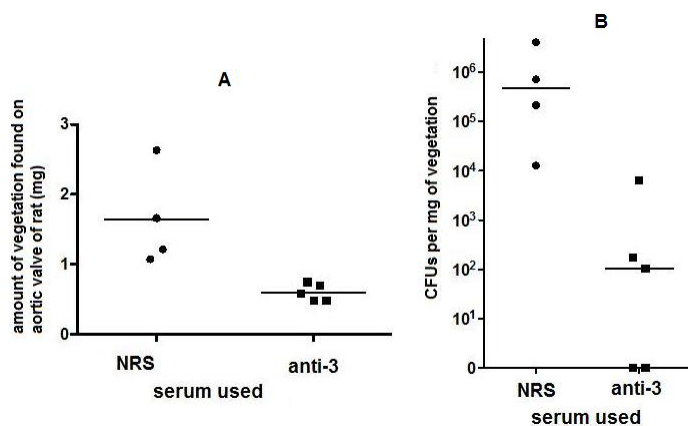
**Figure 5.** Crossreactivity of the serum: Opsonic killing of *E. Faecalis*, *E. Faecium* and *S. Aureus* by rabbit serum (dilutions 1:10 → 1:5000) raised against conjugate 3.



**Figure 6.** Opsonic killing of *E. Faecalis* (left) and *E. Faecium* (right) by rabbit anti-3 at dilutions up to 1:200.000.

The next step of the immunological evaluation of conjugate 3 comprised an *in vivo* passive immunization experiment using the serum raised against this conjugate. To this end, a modification of a rat endocarditis model, described originally by Lee *et al.* to measure the protective properties of antiserum raised against *S. aureus* type 5 capsular polysaccharide, was used.<sup>20</sup> Rats were treated with normal rabbit serum (NRS) or anti-3 serum 48 and 24h before they were challenged intravenously with *E. faecalis*. Four days after the infection, rats were euthanized and the total weight of bacterial vegetation on the aortic valve determined. In addition, the amount of colony

forming units (CFUs) per mg of the harvested vegetation was determined. It was found that the weight of endocarditic vegetation present on the aortic valves of the rats receiving NRS was between 1 and 3 mg (see Figure 7A). The total weight of



**Figure 7.** Effects of anti-3 serum in a rat endocarditis model. A: Bacterial matter (in mg) found on the murine aortic valves immunized with either NRS or anti-3 serum. B: CFUs found in the corresponding vegetation (CFUs/mg).

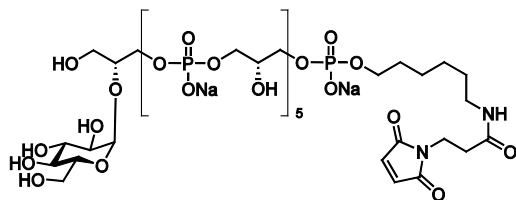
vegetations found on the valves of rats immunized with the anti-3 serum was significantly less (~0.5 mg). The potency of the serum was also revealed by the significant reduction of CFUs present in the bacterial vegetation: 10<sup>4</sup>-10<sup>7</sup> CFUs per mg vegetation were present in rats receiving NRS, whereas bacterial vegetations in the anti-3 immunized rats contained 10<sup>1</sup>-10<sup>4</sup> CFUs per mg on average (see Figure 7B).<sup>21</sup>

## Conclusion

Carbohydrate substituted glycerol TA hexamers **1** and **2** were successfully attached to BSA (**8**) using thiol-maleimide chemistry resulting in conjugates **3** and **4**, respectively. Conjugate **3** showed promising results in initial immunological evaluation experiments and it was shown subsequently that rabbit serum raised against conjugate **3** was able to kill *E. faecalis* in an OPA. In addition, the serum was able to opsonize individual bacterial strains of two other Gram-positive species (*S. aureus* and *E. faecium*). Notably, the activity of the serum against *E. faecium* was especially high and it was shown that the serum could be diluted 160.000-fold before a significant drop in activity resulted. Passive immunization with the rabbit anti-3 serum protected rats against *E. faecalis* in an endocarditis model. The initial immunological evaluation of conjugate **3** indicates that it is a promising lead candidate for a semi-synthetic vaccine against *E. faecalis* and possibly also as a broad-spectrum vaccine directed against several other Gram-positive species. Notably, the results described here show that a relatively small hexameric fragment **1** can be used to elicit an immune response against native LTA. This bodes well for the future development and large scale production of a semi-synthetic synthetic TA vaccine. The next step in the evaluation of conjugate **3** will comprise its evaluation in an active immunization setting.

## Experimental section

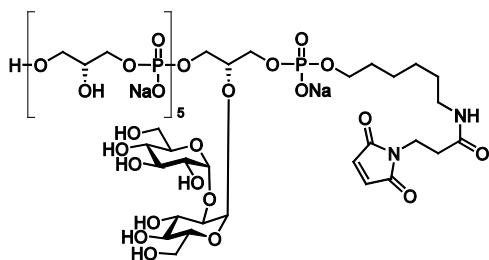
**General procedures:**  $^{31}\text{P}$ ,  $^1\text{H}$ , and  $^{13}\text{C}$  NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively).  $^1\text{H}$ -NMR spectra were recorded in  $\text{D}_2\text{O}$  with chemical shift ( $\delta$ ) relative to HDO (4.755 ppm) at room temperature.  $^{31}\text{P}$  spectra were measured with chemical shift relative to 85%  $\text{H}_3\text{PO}_4$  (external standard) and  $^{13}\text{C}$ -NMR spectra were recorded with chemical shift relative to TMS (external standard). High resolution mass spectra (HRMS) were recorded by direct injection (2  $\mu\text{l}$  of a 2  $\mu\text{M}$  solution in water/acetonitrile; 50/50; v/v and 10mM ammonium formate) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode [source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275  $^\circ\text{C}$ ] with resolution  $R = 60000$  at  $m/z$  400 (mass range  $m/z = 150\text{--}2000$ ) and dioctylphthalate ( $m/z = 391.28428$ ) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). Analysis of the conjugates (**3**, **4**) comprised of sugar quantification<sup>22</sup> (phenol/sulfuric acid assay, using TAs **1** and **2**, respectively, as external standards), protein quantification ( $\text{UV}_{280}$  absorption, using BSA as an external standard) and SDS-PAGE analysis. SDS-PAGE was performed using 7.5% polyacrylamide gels on which BSA (**8**, 300 and 600ng), thiolated BSA (**10**, 500 and 1000 ng), the conjugate (**3** or **4**, 500–2500ng) and Dual Color Marker mixture (containing 37, 50, 75, 100, 150 and 250 kDa markers) were applied after boiling in sample buffer for 5 minutes. Stacking onto the running gel was performed in 30 minutes at 90V. Run time 90–120 minutes at 120V. Visualization of proteins and conjugates was achieved after staining overnight with Coomassie Brilliant Blue followed by washing three or four times with milliQ over a period of 24 hrs.



### Glucosylated glycerol phosphate hexamer maleimide (**6**)

To a solution of TA **1** (7.0 mg, 5.3  $\mu\text{mol}$ ) in a mixture of saturated aqueous  $\text{NaHCO}_3$  (100  $\mu\text{l}$ ) and  $\text{H}_2\text{O}$  (300  $\mu\text{l}$ ) was added *N*-succinimidyl-3-maleimido propionate **5** (28.4 mg, 107  $\mu\text{mol}$ , dissolved in 800  $\mu\text{l}$  3/2 MeCN/1,4-dioxane). After stirring

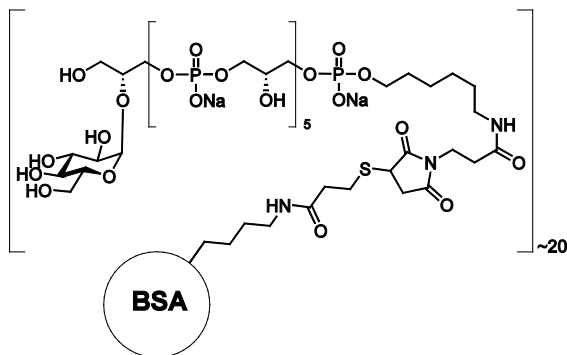
overnight at RT, the mixture was diluted with  $\text{H}_2\text{O}$  (~5 ml) and washed with EtOAc (20 ml). The organic layer was extracted once with 5 ml of  $\text{H}_2\text{O}$  and the combined aqueous layers concentrated under reduced pressure until ~1 ml was left. This solution was applied to a size exclusion column (Sephadex HW40, eluent: 0.05 M  $\text{Et}_3\text{NHOAc}$ ) after which the fractions corresponding to the TA fragment were evaporated and, subsequently, lyophilized three times. The crude product (in ~0.5 ml  $\text{H}_2\text{O}$ ) was then eluted through a small column containing Dowex  $\text{Na}^+$  cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in  $\text{H}_2\text{O}$ , flushed with  $\text{H}_2\text{O}$  and MeOH before use). After concentration *in vacuo* the purified product was coevaporated three times with 1% AcOH (~5 ml) in order to regain the maleimide in the non-hydrolyzed form. Finally, after lyophilization TA maleimide **6** (5.3 mg, 3.6  $\mu\text{mol}$ , 67%) was obtained as an amorphous off-white solid.  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ , hydrolyzed maleimide):  $\delta = 1.2 - 1.3$  (5P), 1.4 (1P);  $^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ , hydrolyzed maleimide):  $\delta = 1.23 - 1.33$  (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.39 - 1.45 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.52 - 1.58 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.38 (t, 2H,  $J = 6.6$  Hz,  $\text{CH}_2$  maleimidopropionyl), 3.09 (t, 2H,  $J = 6.9$  Hz,  $\text{CH}_2\text{-N}$  hexylspacer), 3.33 (at, 1H,  $J = 9.5$  Hz, H-4), 3.39 (t, 2H,  $J = 6.6$  Hz,  $\text{CH}_2\text{-N}$  maleimidopropionyl), 3.46 (dd, 1H,  $J = 3.9$  Hz, 9.9 Hz, H-2), 3.65 - 4.00 (m, 36H, H-3, H-5, H-6, H-6',  $\text{CH}_2\text{-O}$  hexylspacer, 6 x CH glycerol, 12 x  $\text{CH}_2$  glycerol), 5.10 (d, 1H,  $J = 3.8$  Hz, H-1), 5.83 (d, 1H,  $J = 12.2$  Hz, CH maleimide), 6.24 (d, 1H,  $J = 12.3$  Hz, CH maleimide); HRMS:  $\text{C}_{37}\text{H}_{72}\text{N}_2\text{O}_{39}\text{P}_6 + \text{H}^+$  requires 1355.2211, found 1355.2225.



### Kojibiosylated glycerol phosphate hexamer maleimide (7)

To a solution of kojibiosyl TA **2** (10.0 mg, 6.42  $\mu\text{mol}$ ) in a mixture of saturated aqueous  $\text{NaHCO}_3$  (300  $\mu\text{l}$ ) and  $\text{H}_2\text{O}$  (300  $\mu\text{l}$ ) was added *N*-succinimidyl-3-maleimido propionate **5** (23.3 mg, 87.4  $\mu\text{mol}$ , dissolved in 1.0 ml 3/2 MeCN/1,4-dioxane). After stirring overnight at RT, the mixture was diluted with  $\text{H}_2\text{O}$  (~5 ml) and washed with EtOAc (20 ml). The

organic layer was extracted once with 5 ml of  $\text{H}_2\text{O}$  and the combined aqueous layers concentrated under reduced pressure until ~1 ml was left. This solution was applied to a size exclusion column (Sephadex HW40, eluent: 0.05 M  $\text{Et}_3\text{NHOAc}$ ) after which the fractions corresponding to the TA fragment were evaporated and, subsequently, lyophilized three times. The crude product (in ~0.5 ml  $\text{H}_2\text{O}$ ) was then eluted through a small column containing Dowex  $\text{Na}^+$  cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in  $\text{H}_2\text{O}$ , flushed with  $\text{H}_2\text{O}$  and MeOH before use). After concentration *in vacuo* the purified product was coevaporated three times with 1% AcOH (~5 ml) in order to regain the maleimide in the non-hydrolyzed form. Finally, after lyophilization TA maleimide **7** (9.11 mg, 5.52  $\mu\text{mol}$ , 86%) was obtained as an amorphous off-white solid.  $^{31}\text{P}$  NMR (161.7 MHz,  $\text{D}_2\text{O}$ , hydrolyzed maleimide):  $\delta$  = 0.9 (1P), 1.1 (1P), 1.2 - 1.3 (3P), 1.3 (1P);  $^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ , hydrolyzed maleimide):  $\delta$  = 1.25 - 1.33 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.41 - 1.47 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.54 - 1.59 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.39 (t, 2H,  $J$  = 6.6 Hz,  $\text{CH}_2$  maleimidopropionyl), 3.10 (t, 2H,  $J$  = 6.9 Hz,  $\text{CH}_2$ -N hexylspacer), 3.34 - 3.42 (m, 4H, 2 x H-4,  $\text{CH}_2$ -N maleimidopropionyl), 3.49 - 3.56 (m, 2H, CHH glycerol, H-2), 3.59 - 3.63 (m, 2H, CHH glycerol, H-2), 3.67 - 4.01 (m, 37H, 5 x CH glycerol, 11 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$ -O hexylspacer, 2 x H-3, 2 x H-5, 4 x H-6), 4.10 - 4.15 (m, 1H, CH glycerol), 5.10 (d, 1H,  $J$  = 3.7 Hz, H-1), 5.39 (d, 1H,  $J$  = 3.5 Hz, H-1), 5.84 (d, 1H,  $J$  = 12.3 Hz, CH maleimide), 6.25 (d, 1H,  $J$  = 12.3 Hz, CH maleimide); HRMS (hydrolyzed form):  $\text{C}_{43}\text{H}_{84}\text{N}_2\text{O}_{45}\text{P}_6 + \text{Na}^+$  requires 1557.2664, found 1557.2677.

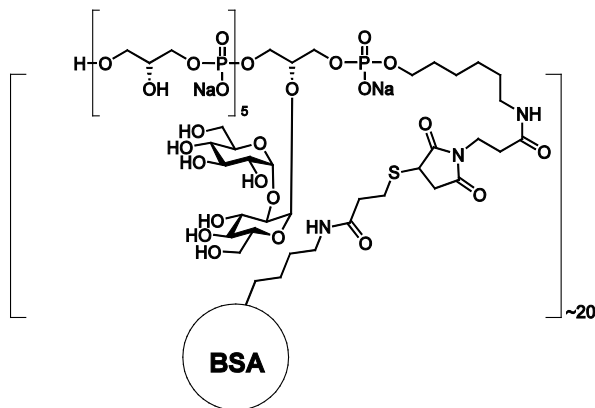


### Glucosyl TA-BSA conjugate(3)

To a solution of freshly prepared<sup>18</sup> thiolated BSA (**10**, 68 nmol protein, max 2.9  $\mu\text{mol}$  thiol, ~4.7 mg) in phosphate buffer saline (PBS, pH 7.2, 1.65 ml) was added TA **6** (5.2 mg, 3.5  $\mu\text{mol}$ , in 400  $\mu\text{l}$  PBS). After stirring for 20 hrs at RT, 3-maleimidopropionic acid (0.65 mg, 3.8  $\mu\text{mol}$ , in 150  $\mu\text{l}$  PBS) was added. After overnight reaction the solution was transferred to a Slide-A-Lyzer dialysis cassette (20 kDa MW cut off, capacity 3 ml) and dialyzed overnight

versus PBS (3l) and milliQ (2 x 3l, overnight). The resulting solution was lyophilized giving the target glucosylated hexaglycerol phosphate-BSA conjugate **3** (~8 mg) as an amorphous white solid. The protein:sugar ratio was found ~1.5:1 and was determined by UV<sub>280</sub> and a phenol/ $\text{H}_2\text{SO}_4$  assay as described above. SDS-PAGE analysis was performed as described in the general procedures and shows a broad band around 95 kDa (see Figure 2, gel A, lane 8,9). These combined results indicate the presence of ~20 TA fragments per molecule of BSA. Details SDS-

PAGE (see also figure 2, gel A); lane 1: BSA (600ng), lane 2: dual color mix (37, 50, 75, 100, 150, 250 kDa markers), lane 3: BSA (300 ng) and **10** (500 ng), lane 4: **10** (500 ng), lane 5: **10** (1000 ng), lane 6: **10** (500 ng), lane 7: dual color mix, lane 8: **3** (1200 ng), lane 9: **3** (2400 ng).



#### Kojibiosyl TA-BSA conjugate(4)

To kojibiosyl TA **7** (3.4 mg, 2.0  $\mu$ mol) was added a solution of freshly prepared<sup>18</sup> thiolated BSA (**10**, 46 nmol protein, max 2.0  $\mu$ mol thiol, ~3.1 mg) in phosphate buffer saline (PBS, pH 7.2, 1.39 ml). After stirring for 20 hrs at RT, 3-maleimidopropionic acid (0.34 mg, 2.0  $\mu$ mol, in 100  $\mu$ l PBS) was added. After overnight reaction the solution was transferred to a Slide-A-Lyzer dialysis cassette (20 kDa MW cut off, capacity 3 ml) and dialyzed overnight versus PBS (3l)

and milliQ (2 x 3l, overnight). The resulting solution was lyophilized giving the target kojibiosylated hexaglycerol phosphate-BSA conjugate **4** (~5 mg) as an amorphous white solid. The protein:sugar ratio was found ~1.5:1 and was determined by UV<sub>280</sub> and a phenol/H<sub>2</sub>SO<sub>4</sub> assay as described above. SDS-PAGE analysis was performed as described in the general procedures and shows a broad band around 100 kDa (see Figure 2, gel B, lane 5-7). These combined results indicate the presence of ~20 kojibiosyl TA fragments per molecule of BSA. Details SDS-PAGE (see also figure 2, gel B); lane 1: BSA (600ng), lane 2: BSA (300 ng) and **10** (500 ng), lane 3: **10** (600 ng), lane 4: **10** (1000 ng), lane 5: **10** (600 ng) and **4** (600 ng), lane 6: **4** (600 ng), lane 7: **4** (1200 ng), lane 8: dual color mix (37, 50, 75, 100, 150, 250 kDa markers).

**Rabbit immunization**<sup>6</sup>: Rabbits were immunized according to literature precedents using 2 injections of 100  $\mu$ g s.c. of either BSA or conjugate **3** or **4**. in incomplete Freund adjuvant, followed by 9 i.v. injections over 3 weeks. All serum samples were heat-inactivated to remove complement activity. For all experiments, serum from the final bleed of the rabbit was used.

**Opsonophagocytic assays**<sup>6</sup>: These assays were performed as described by Theilacker *et al*. White blood cells (WBCs) were collected by dextran sedimentation from fresh human blood collected from healthy adult volunteers. Baby rabbit serum (Cedarlane Laboratories, Hornby, Ontario, Canada) diluted 1:15 in Roswell Park Memorial Institute medium (RPMI)-fetal bovine serum was used as the complement source. Unless stated otherwise, bacteria (*E. faecalis* 12030, *E. faecium* E1162, *S. aureus* MW2) grown overnight on tryptic soy agar were adjusted to an optical density at 650 nm (OD<sub>650</sub>) of 0.1 with fresh TSB and allowed to grow to an OD<sub>650</sub> of 0.4. For comparison of opsonophagocytosis of *S. aureus* strains in different growth phases, an additional culture was grown overnight to late stationary phase, and the bacterial concentration was adjusted to an OD of 0.4 before the assay. Equal volumes of bacterial suspension (2.5 x 10<sup>7</sup> per mL), leukocytes (2.5 x 10<sup>7</sup> per mL), complement source (1.7% final concentration), and immune rabbit serum (anti-BSA, anti-**3** or anti-**4**) at the dilutions indicated in figures 3-6 were combined and incubated on a rotor rack at 37 °C for 90 minutes. After incubation, live bacteria were quantified by agar culture of serial dilutions. Controls included tubes lacking serum, tubes containing complement but lacking serum and WBCs, and tubes containing serum and WBCs but lacking complement. For all bacterial strains tested in the study, no significant killing (<5%)

was observed in the presence of complement alone. The percentage of killing was calculated by determining the mean number of colony-forming units (CFUs) in tubes without WBCs to the number of CFUs in tubes with serum samples, leukocytes, complement, and bacteria.

**Opsonophagocytic inhibition assays:** anti-3 serum was diluted to a concentration of 1:1000 (final concentration) giving ~90% killing in the OPA. This antiserum was incubated at 4 °C for 60 minutes with 0.8-100 µg/mL of inhibitor. After this incubation step, the OPA was continued as described above. The percentage of inhibition of killing was calculated by determining the ratio of the CFUs surviving in the tubes with inhibitor to the CFUs surviving in the tubes without inhibitor.

**Rat endocarditis experiments:** The experiments were performed following a procedure described by Lee *et al.*<sup>20</sup> using either normal rabbit serum (control) or anti-3 serum.



## References

1. Lode, H.M. *Clin. Microbiol. Infect.* **2009**, *15*, 212-217.
2. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Sava, I.; Ye, L.; Bychowska, A.; Holst, O.; Huebner, J. *Plos One* **2011**, *6*, e17839.
3. Theilacker, C.; Krueger, W.A.; Kropec, A.; Huebner, J. *Vaccine*, **2004**, *22S*, 31-38.
4. Sava, I.G.; Heikens, E.; Huebner, J. *Clin. Microbiol. Inf.* **2010**, *16*, 533-540.
5. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Fabretti, F.; Sange, T.; Holst, O.; Huebner, J. *Infect. Immun.* **2006**, *74*, 5703-5712.
6. Theilacker, C.; Kropec, A.; Hammer, F.; Sava, I.; Wobser, D.; Sakinc, T.; Codée, J.D.C.; Hogendorf, W.F.J.; van der Marel, G.A.; Huebner, J. *J. Infect. Dis.* **2012**, *205*, 1076-1085.
7. Hogendorf, W.F.J.; Meeuwenoord, N.; Overkleeft, H.S.; Filippov, D.V.; Laverde, D.; Kropec, A.; Huebner, J.; van der Marel, G.A.; Codée, J.D.C. *Chem. Commun.* **2011**, *47*, 8961-8963.
8. Hecht, M.-L.; Stallforth, P.; Varón Silva, D.; Adibekian, A.; Seeberger, P.H. *Curr. Opin. Chem. Biol.* **2009**, *13*, 354-359.
9. Vliegenthart, J.F.G. *FEBS Lett.* **2006**, *580*, 2945-2950.
10. Hermanson, G.T. *Bioconjugate Techniques*, Academic Press: San Diego, USA, **1996**.
11. Giebink, G.S.; Koskela, M.; Vella, P.P.; Harris, M.; Le, C.T. *J. Infect. Dis.* **1993**, *167*, 347-355.
12. Whitney, C.G.; Farley, M.M.; Hadler, J.; Harrison, L.H.; Bennett, N.M.; Lynfield, R.; Reingold, A.; Cieslak, P.R.; Pilishvili, T.; Jackson, D.; Facklam, R.R.; Jorgensen, J.H.; Schuchat, A.; *N. Engl. J. Med.* **2003**, *348*, 1737-1746.
13. Lin, F.Y.; Ho, V.A.; Khiem, H.B.; Trach, D.D.; Bay, P.V.; Thanh, T.C.; Kossaczka, Z.; Bryla, D.A.; Shiloach, J.; Robbins, J.B.; Schneerson, R.; Szu, S.C. *N. Engl. J. Med.* **2001**, *344*, 1263-1269.
14. Pozsgay, V.; Chu, C.Y.; Pannell, L.; Wolfe, J.; Robbins, J.B.; Schneerson, R. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5194-5197.
15. Campbell, W.N.; Hendrix, E.; Cryz, S. Jr.; Cross, A.S. *Clin. Infect. Dis.* **1996**, *23*, 179-181.
16. Lancaster, L.; Saydam, M.; Markey, K.; Ho, M.M.; Mawas, F. *Vaccine* **2011**, *29*, 3213-3221.
17. Verez-Bencomo, V.; Fernández-Santana, V.; Hardy, E.; Toledo, M.E.; Rodríguez, M.C.; Heynngnezz, L.; Rodriguez, A.; Baly, A.; Herrera, L.; Izquierdo, M.; Villar, A.; Valdés, Y.; Cosme, K.; Deler, M.L.; Montane, M.; Garcia, E.; Ramos, A.; Aguilar, A.; Medina, E.; Toraño, G.; Sosa, I.; Hernandez, I.; Martínez, R.; Muzachio, A.; Carmenates, A.; Costa, L.; Cardoso, F.; Campa, C.; Diaz, M.; Roy, R. *Science* **2004**, *305*, 522-525.
18. Bundle, D.R. *Nat. Chem. Biol.* **2007**, *3*, 605-606.
19. Fernández-Santana, V.; González-Lio, R.; Sarracent-Pérez, J.; Verez-Benzomo, V. *Glycoconj. J.* **1998**, *15*, 549-553.
20. Lee, J.C.; Park, J.S.; Shepherd, S.E.; Carey, V.; Fattom, A. *Infect. Immun.* **1997**, *65*, 4146-4151.
21. Manuscript in preparation.
22. Fournier, E. *Current Protocols in Food Analytical Chemistry*, John Wiley & Sons Inc.: New York, USA, **2001**.

## Chapter 7

---

### Summary and Future Prospects

This thesis describes the development of synthetic methodologies to acquire structurally defined fragments of teichoic acids (TAs). TAs are an important class of phosphodiester containing biopolymers that occur in the cell wall of Gram-positive bacteria.<sup>1-5</sup> The structure of TAs mostly consists of linear polymers of alditol (glycerol, ribitol) phosphates, non-stoichiometrically substituted with D-alanyl and carbohydrate moieties. The TA structure is related to the type of bacteria and is dependent on the conditions these encounter. TAs not only take part in vital functions of the bacteria but also are immunologically active. In the introductory chapter the discovery, the structural classification, as well as physiological and immunological aspects of TAs is briefly discussed. In addition, selected examples of reported syntheses to TA fragments, with a focus on phosphor chemistry are presented. The synthetic efforts described in this thesis are mainly directed to TAs originating from *Enterococcus faecalis*. This normally harmless commensal has become a considerable threat in hospitals as growing antibiotic resistance makes this species difficult to treat.<sup>6-10</sup> In collaboration with the group of Huebner, specialists on the pathogenesis, treatment, and prevention of enterococcal infections, the prepared TAs are immunologically evaluated.

**Chapter two** describes the solution phase synthesis of a spacer containing (kajibiosyl) glycerol hexamer, a striking fragment of a TA from *Enterococcus faecalis*.<sup>9</sup> Benefit was taken from knowledge on oligonucleotide synthesis, indicating that phosphoramidite chemistry is the most efficient procedure for the formation of phosphodiesters and the dimethoxytrityl group is most appropriate as temporary protective group. Suitably protected glycerol and kajibiosyl glycerol phosphoramidite building blocks and an aminohexylphosphoramidite were prepared and implemented in the stepwise elongation of dibenzylglycerol to the target hexamer. Each elongation cycle comprised three reactions: 1) Phosphitylation using a phosphoramidite. 2) Oxidation of the intermediate phosphite triester. 3) Cleavage of the dimethoxytrityl group. Deprotection and purification of the target hexamer proceeded uneventful. The synthetic approach proved efficient and could be performed at a relatively large scale using only a small excess of phosphoramidite reagents.<sup>11</sup>

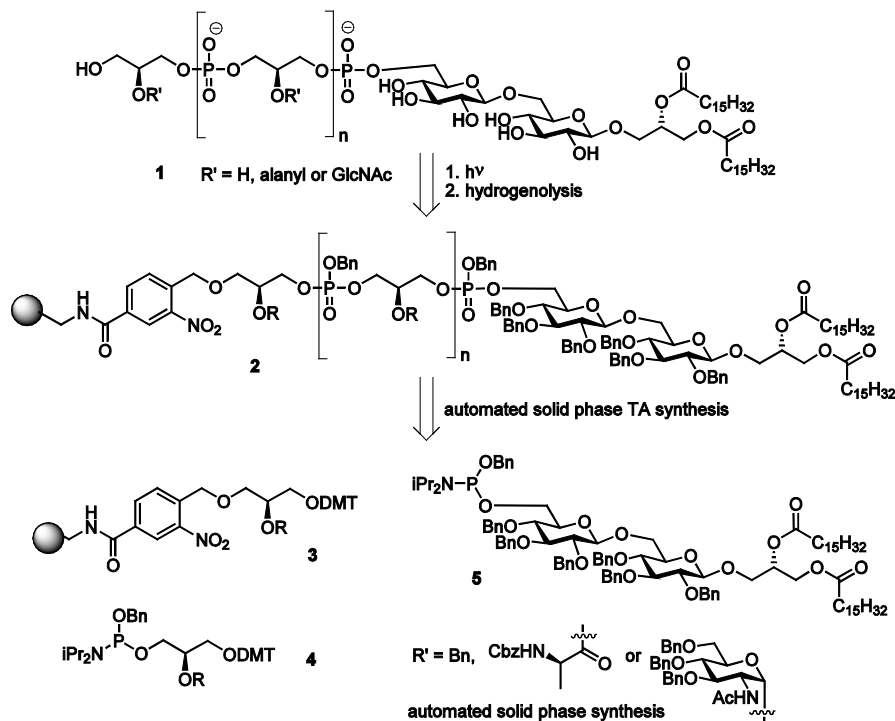
The phosphoramidite chemistry described in **chapter two** could be applied for the development of a solid phase approach, executed with the aid of a DNA synthesizer.

**Chapter three** deals with the construction of a small library of TA fragments using an automated solid phase synthesis approach. The library consists of linear 1,3-glycerol phosphate TAs comprising six, ten, fourteen and twenty residues and two  $\alpha$ -glucosylated hexaglycerol phosphate TAs. The approach proved to be a fast and efficient means to obtain small quantities of a library of TA fragments.<sup>12</sup>

The automated solid phase synthesis protocol described in **chapter three** can potentially give access to all kinds of TAs. Intrinsically base-labile structural entities such as diacyl glycerol, which is the core structure of the glycolipid anchor of all LTAs and alanylated TAs can potentially be made using this approach. However, this excludes the 2-cyanoethyl as a phosphate protecting group and the succinyl linker to the solid-support since these require base treatment in the final stage of the synthesis. The protective group pattern should be adjusted and an example of a retro-synthesis to a fragment of the LTA of *S. aureus* using benzyl protection for the phosphate moieties and a photo-cleavable linker to the solid support is outlined in Scheme 1.

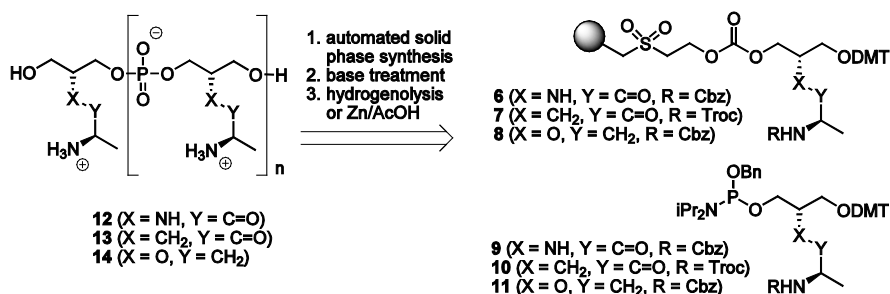
The lability of D-alanyl esters present on TAs makes it difficult to get a clear understanding of the immunomodulatory functions that these moieties possess.<sup>13</sup> Even synthetic TAs, the structure of which is spectroscopically ascertained, can spontaneously degrade when dissolved in the buffer belonging to *in vitro* or *in vivo*

**Scheme 1.** Retrosynthesis of staphylococcal LTA, no significant base is used during the synthesis.



immunological evaluation assays. This problem can possibly be circumvented by the synthesis of TA fragments that are equipped with “stabilized” alanines. Schmidt and co-workers have reported on the use of alanyl amides as replacement of alanyl esters in the synthesis of an analogue of staphylococcal LTA.<sup>14</sup> This structural change, although adding extra potential hydrogen bond donors, minimally affects important properties of teichoic acids such as the overall charge. It is proposed to investigate the optically pure  $\alpha$ -amino ketone as another example of a stabilized D-alanyl ester analogue. In this case the molecules lose a hydrogen bond acceptor for every replacement. A third option would be to omit the carbonyl moiety and incorporate the stereochemically pure 1-(2-amino)-propyl ether as an alanine mimetic. In this case the molecule loses an H-bond acceptor but gains more flexibility. In Scheme 2, a retrosynthetic approach to these TA mimetics is presented. In order to avoid potential epimerization of the alanyl C-2, it is recommended to use a linker to the solid support that is sufficiently base labile. Alternatively, the photo-cleavable linker, the structure of which is depicted in Scheme 1, can be amended for this purpose.

**Scheme 2.** Retrosynthesis of stabilized D-alanylated TA mimetics **12–14**.



**Chapter four** informs on a new light fluoruous approach to the synthesis of a small library of TA fragments, having a terminal phosphate monoester. Glycerol TAs up to a length of a dodecamer and several hexamers containing (amino)glucosyl moieties, as present in TAs of several strains of bacteria, were efficiently obtained via this technique. The molecules were built up from a base labile perfluorooctylpropyl sulfonyl ethyl (F-Pse) scaffold, which concomitantly served as a phosphate protecting group. This method bears the advantages of the solution phase method (**chapter two**), such as the scale of the reaction allowing the production of multimilligram amounts of target TAs and the small excess of expensive phosphoramidite building blocks. In addition, the highly efficient fluoruous solid phase extraction (F-SPE) purification step, which is performed after each elongation cycle ensures a significantly reduced purification time.<sup>15</sup>

**Chapter five** reports on an alternative light fluoruous approach to three glucosylated TA hexamers. In order to obtain TAs provided with a terminal hydroxyl function instead of a phosphate monoester, a base labile fluoruous succinyl linker was developed. In addition, the synthesis towards a more acid stable tetrabenzylglucosyl glycerol phosphoramidite is described. This building block provided with acid stable

protecting groups allowed the introduction of the (glucosyl)glycerol moiety into the TA backbone at an earlier stage of the synthesis.<sup>16</sup>

The chemical research described in **chapters two, three, four** and **five** can be summarized as the development of three distinctive synthetic approaches to TA fragments. These three synthetic approaches: solution phase (**chapter two**), automated solid phase (**chapter three**) and fluorous phase (**chapters four/five**) all have their advantages and disadvantages, which are summarized in Table 1. From the summary drafted in this Table one can envisage that for the development and optimization of the applied chemistry the solution phase approach suits best. For a small-scale synthesis of a library of TA fragments to screen the initial biological properties such as their antigenic potency, the automated solid phase approach is the most suitable method. If screening experiments make available a lead compound provided with an interesting immunological profile, larger quantities of this TA fragment are required for additional immunological experiments. The TA fragment can in that case be obtained on a larger scale using one of the light fluorous approaches (**chapter four/five**), which allows a more rapid and efficient synthesis compared to regular solution phase chemistry (**chapter two**).

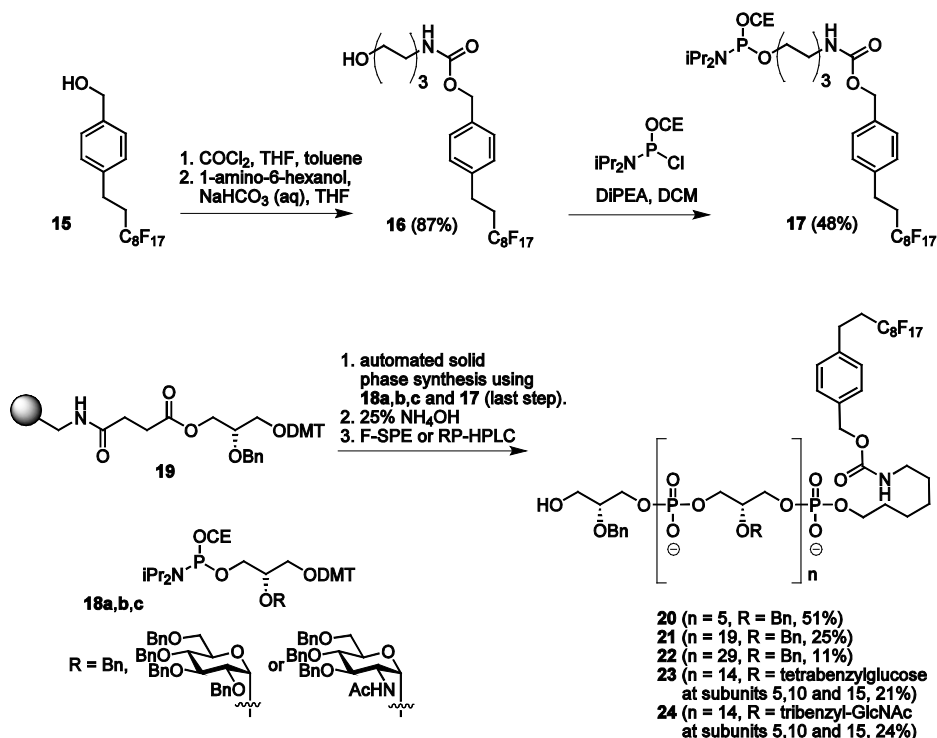
**Table 1.** Relative valuation of the three approaches on several practical elements.  
(+ = good, +/- = reasonable, - = poor)

	Solution phase	Automated solid phase	Light fluorous
Max . work scale	+ (>1 mmol)	- (10-15 $\mu$ mol)	+ (>1 mmol)
Equivalent of phosphoramidite needed for full conversion	+ (1.3-3)	- (5-10)	+ 1.2-3
Maximum length (glycerol phosphate subunits)	- (6)	+ (>20)	+/- (>12)
Purification	- (silica gel)	+ (RP-HPLC or Q-sepharose)	+ (F-SPE)
Total synthesis time	- (weeks)	+ (hours)	+/- (days/weeks)

It was envisioned that the automated solid phase TA synthesis could be improved by amending the purification method with a fluorous tagging procedure.<sup>17-19</sup> This procedure comprises the installation of a suitable fluorous tag in the final elongation step of the automated synthesis to TAs. Because deletion sequences have been capped (acetylated) throughout the assembly process, only the target fragment will be equipped with the fluorous tag. Cleavage from the solid support and removal of the base-labile protecting groups provides a crude mixture, in which the semi-protected

target TA is the sole fluororous compound, allowing an efficient purification by F-SPE or F-HPLC. To test the viability of this purification procedure in TA synthesis, fluororous benzyl carbamate<sup>20</sup> protected aminohexyl phosphoramidite (**17**) was prepared from fluororous benzyl alcohol (**15**) in three steps (see Scheme 3). A small library of TA fragments, in which this fluororous tag was appended, was made. The library consisted of glycerol phosphates hexamer **20**, 20-mer **21**, 30-mer **22**, and two 15-mers, bearing three  $\alpha$ -glucosyl or  $\alpha$ -*N*-acetyl glucosamine residues (**23**, **24**). F-SPE proved to be an efficient purification method for hexamer **20**, although it was observed that the difference in affinity for the F-SPE column of **20** with respect to its by-products was small. Semi-protected hexamer **20** could be obtained in 51% yield. In case of 20-mer **21**, the F-SPE purification of the crude product was significantly less effective, as 20-mer **21** partially co-eluted with non-fluororous by-products. A second F-SPE purification step of the mixed fractions was required to afford the pure target **21** in 25% yield. In the case of 30-mer glycerol phosphate **22**, no separation between the fluororous and non-fluororous products was obtained with F-SPE, indicating the limit of the F-SPE purification method. To effect the purification of **22** the potency of a fluororous HPLC purification was explored. Interestingly, with this technique no significant improvement in separation of the fluororous 30-mer (**22**) from the by-products was

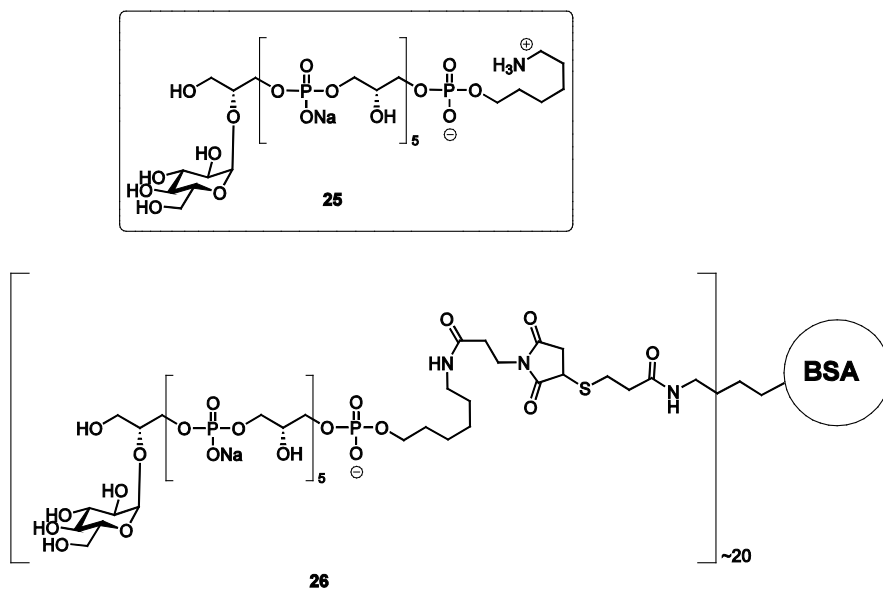
**Scheme 3.** Automated solid phase synthesis of TA fragments using a fluororous tagging strategy.



observed compared to a normal RP-HPLC (C18) purification. Apparently, the lipophilicity of the fluororous tag in these charged, semi-protected, glycerol phosphates has a larger effect on the separation than the fluororous affinity. Purification of the 30-mer with regular preparative RP-HPLC (C18) gave pure **22** in 11% yield. The glycosylated 15-mers **23** and **24** were also purified with preparative RP-HPLC. The relatively lipophilic benzylated carbohydrate residues in 15-mers **23** and **24**, led to significant peak broadening during RP-HPLC analysis using a C18 column. When the RP-HPLC purification was performed with a preparative C4 column the two semi-protected TA 15-mers were isolated in adequate yields: 21% for **23**, and 24% for **24**, respectively. These results indicate that the fluororous tagging strategy is viable for the synthesis of TA fragments. For the smaller fragments, the target compounds can be purified by exploiting the fluororous character of the TAs, where purification of the longer fragments hinges on the extra lipophilicity of the fluororous benzyl group attached to the targets, as a RP-HPLC-purification handle.

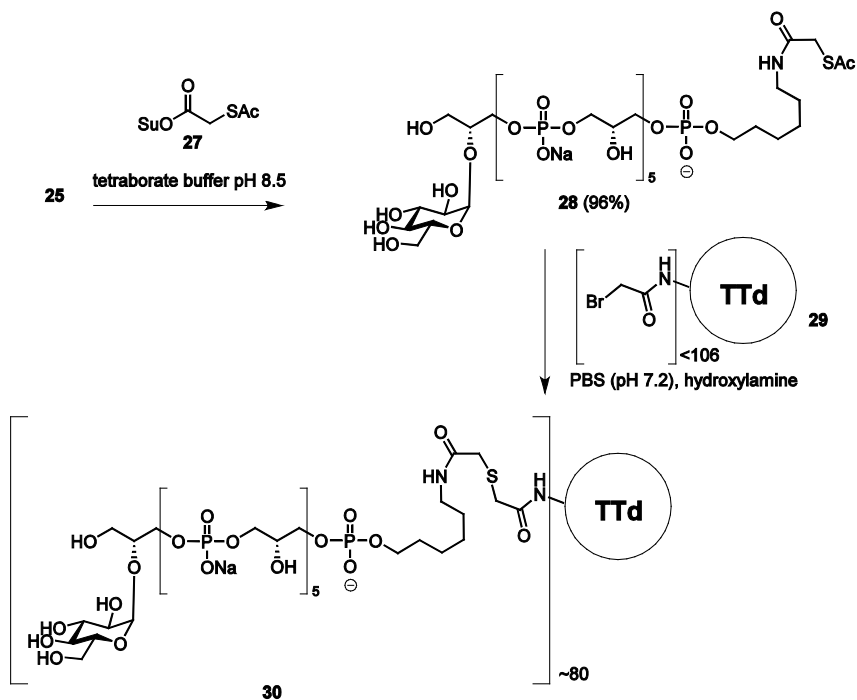
The TA fragments described in **chapters two, three, four** and **five** were tested on their antigenicity in an opsonophagocytic inhibition assay (OPIA). The antigenicity of the compounds depended on their length, and the non-substituted 20-mer inhibited the killing of *E. faecalis* more efficiently than the shorter non-substituted glycerol phosphates. Surprisingly, the fragments containing an  $\alpha$ -glucosyl moiety, which is a substitution not found in native enterococcal LTA, showed even better inhibitory activity in the OPIA (see **chapter three**) than the 20-mer. Surprisingly, the TA fragments containing a phosphate monoester functionality (**chapter four**) all showed low activity and the bis-glucosylated hexaglycerol phosphate (**chapter five**) was less active than its monoglucosylated counterpart (structure **25**, Figure 1). The outcome of these studies indicate that the activity of the TA fragments critically depends on the substitution pattern and that more structure activity studies are warranted to arrive at a TA fragment with an optimal immunological profile.<sup>10,12</sup>

**Chapter six** describes the synthesis and immunological evaluation of a conjugate of fragment **25** with bovine serum albumin (BSA), an immunogenic carrier protein. Conjugate **26** was obtained via thiol maleimide chemistry and the conjugate contained ~20 TAs per protein (see Figure 1).<sup>21</sup> Serum obtained from rabbits, immunized with this conjugate, showed high opsonic activity against *E. faecalis*. Notably, two strains of different Gram-positive pathogens (*Enterococcus faecium* and *Staphylococcus aureus*) were also opsonized this rabbit anti-**26** serum. The opsonic activity against *E. faecium* was surprisingly high, as the antiserum could be diluted ~160.000 fold before the killing was reduced. In an *in vivo* endocarditis model it was shown that rats could successfully be passively immunized against *E. faecalis* using the rabbit antiserum raised against conjugate **26**.<sup>22</sup> A next step in the evaluation of conjugate **26** as an experimental vaccine modality would be to test the possibility of active immunization.



**Figure 1.** Hexamer **25**, the TA fragment with the highest inhibitory activity, and its BSA conjugate (**26**).

**Scheme 4.** Synthesis of TA-TTd conjugate **30**.





When TA-BSA conjugate **26** proves successful in the additional immunological evaluation, it is needed to replace BSA by a protein carrier that is more suitable for clinical studies. One of these carrier proteins is tetanus toxoid (TTd), which is used in several conjugate vaccines. A notable example is the Quimihib®-vaccine, a TTd-conjugate of the (synthetic) capsular polysaccharide of *Haemophilus influenza*.<sup>21</sup> In anticipation, TA fragment **25** was equipped with a (2-thioacetyl)acetyl moiety using the corresponding hydroxysuccinimide ester (**27**) to give modified TA **28** in 96% yield (see Scheme 4). The thiol functionality in **28** was liberated in a buffer containing hydroxylamine, and coupled *in situ* to bromoacetylated-TTd (**29**) as described earlier by Hoogerhout and co-workers.<sup>23,24</sup> An H<sub>2</sub>SO<sub>4</sub>/phenol colorimetric assay<sup>25</sup> of the resulting conjugate (**30**) indicated the presence of carbohydrates on the protein and a protein/sugar ratio of ~1.25/1 (~80 TAs per TTd). The immunological evaluation of this conjugate is currently in progress.

In conclusion, this thesis describes the development of three distinctive synthetic methodologies to well defined TA fragments. The solution phase, automated solid phase and light fluoruous approaches all have their specific merits and can be amended for different purposes. The synthetic TA fragments have been evaluated on their antigenicity and the most potent compound has been conjugated to a protein carrier. The TA-protein conjugate shows promising results in initial *in vitro* and *in vivo* immunological assays and can be used to passively immunize rats against *E. faecalis* an increasingly important Gram-positive threat in healthcare. Further biological studies will indicate whether the conjugate is a suitable candidate for clinical trials.

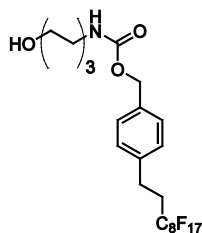
## Experimental section

**General Procedures and Material:** All chemicals (Acros, Fluka, Merck, Schleicher & Schuell, Sigma-Aldrich, Genscript, Fluorous Technologies) were used as received and reactions were carried out dry, under an argon atmosphere, at ambient temperature, unless stated otherwise. Column chromatography was performed on Screening Devices silica gel 60 (0.040-0.063 mm). TLC analysis was conducted on HPTLC aluminium sheets (Merck, silica gel 60, F245). Compounds were visualized by UV absorption (245 nm), by spraying with 20% H<sub>2</sub>SO<sub>4</sub> in ethanol or with a solution of (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>•4 H<sub>2</sub>O 25 g/l and (NH<sub>4</sub>)<sub>4</sub>Ce(SO<sub>4</sub>)<sub>4</sub>•2 H<sub>2</sub>O 10 g/l, in 10% aqueous H<sub>2</sub>SO<sub>4</sub> followed by charring at +/- 140 °C. <sup>31</sup>P, <sup>1</sup>H, and <sup>13</sup>C NMR spectra were recorded with a Bruker AV 400 (161.7, 400 and 125 MHz respectively) or a Bruker DMX 600 (600 and 150 MHz respectively). NMR spectra were recorded in CDCl<sub>3</sub> with chemical shift (δ) relative to tetramethylsilane, unless stated otherwise. When D<sub>2</sub>O was used, <sup>1</sup>H-NMR spectra were recorded with chemical shift relative (δ) to HDO (4.755 ppm), <sup>31</sup>P spectra were measured with chemical shift relative to 85% H<sub>3</sub>PO<sub>4</sub> (external standard) and <sup>13</sup>C-NMR spectra were recorded with chemical shift relative to TMS (external standard). High resolution mass spectra (HRMS) were recorded by direct injection (2 µl of a 2 µM solution in water/acetonitrile; 50/50; v/v and either 0.1% formic acid or 10mM ammonium formate for the oligomers) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 275 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylphthalate (m/z = 391.28428) as a lock mass. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan).

**Procedure for automated solid-phase synthesis, purification and global deprotection of TA oligomers:** Modified polystyrene support (amino PS, Fluka) was loaded with glycerol succinates and the loading was determined (loading: 100  $\mu\text{mol/g}$  CPG) using the method described by Pon.<sup>ref</sup> The automated syntheses were performed on a synthesizer (ÅKTA<sup>TM</sup> oligopilot plus<sup>TM</sup>, GE Healthcare) on a scale of 100-150 mg immobilized **19** (10-15  $\mu\text{mol}$  glycerol derivative) and started off with acidolysis of the dimethoxytrityl ether using 3% dichloroacetic acid in toluene (15 ml, 3 min). After flushing with acetonitrile (5ml, 1 min), the resulting alcohol was reacted with phosphoramidites **18a** (0.2 M in MeCN, 5 eq) and 5-benzylthiotetrazole (BTT, 0.3M in MeCN, 22.5 eq) for 5 min using a cycled flow. After flushing with MeCN (5ml, 1 min), oxidation of the intermediate phosphite was performed using  $\text{I}_2$  (0.05 M in pyridine/ $\text{H}_2\text{O}$  9/1, 2ml, 1 min). A flushing step with MeCN (5ml, 1 min) was followed by a capping step (1 ml of a 1/1 mixture of capping solution A (20 v/v% *N*-methylimidazole in MeCN) and capping solution B (20 v/v%  $\text{Ac}_2\text{O}$ , 15 v/v% 2,6-lutidine in MeCN for 12s). After flushing with MeCN (5ml, 1 min), a detritylation step was performed using the before mentioned cocktail and the molecule was elongated using phosphoramidites **18a,b** or **c** using the same set of reactions (coupling, oxidation, capping, detritylation). The average coupling efficiency was measured by quantitative UV-detection (400 nm) of the dimethoxytrityl cation during each detritylation step. When the desired length was obtained, spacer phosphoramidite **17** (0.2 M in ACN, 2 x 5 eq, 2 x 5 min) was coupled to the PS-TA-oligomer using BTT (0.3M in MeCN, 2 x 22.5 eq) and, subsequently treated with  $\text{I}_2$  (0.05 M in pyridine/ $\text{H}_2\text{O}$  9/1, 2ml, 1 min), before it was released from the solid support using 25%  $\text{NH}_4\text{OH}$  (10ml, 1h, the cyanoethyl groups are concomitantly released at this stage). The solvents were then removed *in vacuo* before the crude oligomer was purified using F-SPE or RP-HPLC.

**F-SPE:** The crude product was taken up in 0.5 ml  $\text{H}_2\text{O}$  and applied to a small column containing fluoroflash<sup>TM</sup> fluorosilica (4g) which was preeluted with  $\text{H}_2\text{O}$ . The column was eluted with  $\text{H}_2\text{O}$  and 10%  $\text{CH}_3\text{CN}$  in  $\text{H}_2\text{O}$  until all the non-fluorous by-products were removed. The fluorosilica product was then eluted from the column with a 2/2/1 mixture of acetonitrile/ $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ . Lyophilization gave the partially protected oligomer of which the integrity and purity was confirmed by LC-MS, HRMS and NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ ) analysis.

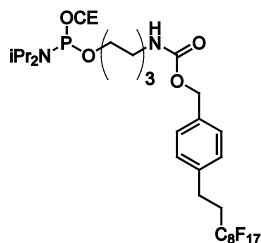
**RP-HPLC:** Gilson preparative HPLC system; column: Alltima C18 or C4, particle size: 5  $\mu\text{m}$ , dimensions: 10/250 mm; eluent: (10 mM  $\text{NH}_4\text{OAc}$  in  $\text{H}_2\text{O}$  for C18 or 25mM TEAA for in  $\text{H}_2\text{O}$  for C4)/MeCN, 9/1  $\rightarrow$  1/9, detection: UV (215 and 254 nm), the fractions containing product were collected and the solvents were removed under reduced pressure. Repeated lyophilization (trice) of the residue gave the partially protected oligomer of which the integrity and purity was confirmed by LC-MS, HRMS and NMR ( $^1\text{H}$ ,  $^{13}\text{C}$ ,  $^{31}\text{P}$ ) analysis.



#### 4-(2-perfluorooctylethyl)benzyl *N*-(6-hydroxyhexyl)carbamate (**16**)

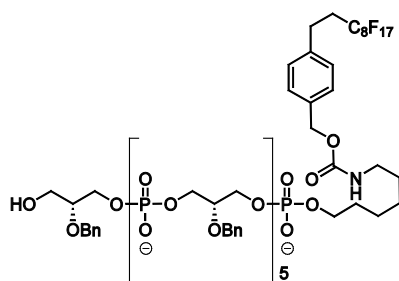
To a cooled (0  $^\circ\text{C}$ ) solution of fluorosilica benzyl alcohol **15** (1.11 g, 2.00 mmol) in THF (20 ml) was added phosgene (2M in toluene, 2.50 ml, 5 mmol). After stirring for 90 minutes the volatiles were removed under reduced pressure and the residue coevaporated with THF (10 ml). The crude chloroformate was dissolved in a mixture of THF (30 ml) and sat. aq.  $\text{NaHCO}_3$  (12 ml) after which 1-amino-6-hexanol (2.34 g, 20 mmol) was added. After stirring overnight the mixture was diluted with  $\text{Et}_2\text{O}$  (300 ml) and washed with brine (2 x 150 ml). The organic layer was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo*. Crystallization ( $\text{EtOAc}/\text{PE}$ ) of the residue afforded the title carbamate (**16**, 1.21 g, 1.74 mmol, 87%) as an amorphous off-white solid.  $^1\text{H}$  NMR (300 MHz):  $\delta$  = 1.30 - 1.41 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 1.47 - 1.60 (m, 4H, 2 x  $\text{CH}_2$  hexylspacer), 2.27

- 2.48 (m, 2H,  $F_{17}C_8CH_2CH_2-$ ), 2.86 - 2.98 (m, 2H,  $F_{17}C_8CH_2CH_2-$ ), 3.11 - 3.21 (m, 2H,  $CH_2-N$  hexylspacer), 3.58 (t, 2H,  $J = 6.5$  Hz,  $CH_2-O$  hexylspacer), 5.06 (s, 2H,  $CH_2$  F-Bn), 5.41 (bs, 1H, NH), 7.22 (d, 2H,  $J = 7.8$  Hz,  $H_{arom}$ ), 7.32 (d, 2H,  $J = 7.9$  Hz,  $H_{arom}$ );  $^{13}C$  NMR (75 MHz):  $\delta = 25.1$ , 25.9, 26.2 (2 x  $CH_2$  hexylspacer,  $F_{17}C_8CH_2CH_2-$ ), 29.6, 32.1 (2 x  $CH_2$  hexylspacer), 32.7 (t,  $J = 22$  Hz,  $F_{17}C_8CH_2CH_2-$ ), 40.7 ( $CH_2-N$  hexylspacer), 61.9 ( $CH_2-O$  hexylspacer), 66.1 ( $CH_2$  F-Bn), 128.3 - 128.4 ( $CH_{arom}$ ), 134.9 ( $C_q$  F-Bn), 138.9 ( $C_q$  F-Bn).



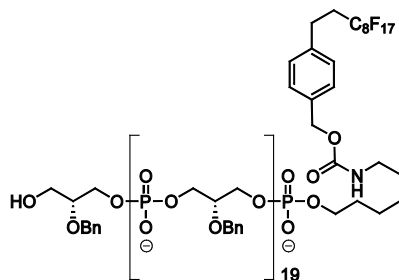
#### 4-(2-perfluorooctylethyl)benzyl *N*-(6-*O*-[*N,N*-diisopropyl]-2-cyanoethyl phosphoramidite)-hexyl)-carbamate (**17**)

To a solution of fluororous alcohol **16** (1.13 g, 1.62 mmol) and DiPEA (0.43 ml, 2.47 mmol) in DCM (32 ml) was added (*N,N*-diisopropyl)-2-cyanoethyl-chlorophosphoramidite (0.452 ml, 2.03 mmol). After stirring 90 min  $H_2O$  (10 ml) was added and the layers separated. The organic layer was washed with  $H_2O$  (10 ml) and brine (10 ml) after which it was dried ( $Na_2SO_4$ ) and concentrated *in vacuo*. Purification of the residue by column chromatography (EtOAc/PE/Et<sub>3</sub>N) gave phosphoramidite **17** (692 mg, 771  $\mu$ mol, 48%) as an oily substance.  $^{31}P$  NMR (161.7 MHz,  $CD_3CN$ ):  $\delta = 148.2$ ;  $^1H$  NMR (400 MHz,  $CD_3CN$ ):  $\delta = 1.16$  - 1.20 (m, 12H, 4 x  $CH_3$  isopropylamino), 1.30 - 1.41 (m, 4H, 2 x  $CH_2$  hexylspacer), 1.44 - 1.51 (m, 2H,  $CH_2$  hexylspacer), 1.56 - 1.62 (m, 2H,  $CH_2$  hexylspacer), 2.35 - 2.49 (m, 2H,  $F_{17}C_8CH_2CH_2-$ ), 2.63 (t, 2H,  $J = 6.0$  Hz,  $CH_2$  cyanoethyl), 2.84 - 2.90 (m, 2H,  $F_{17}C_8CH_2CH_2-$ ), 3.09 (dd, 2H,  $J = 6.6$  Hz, 13.0 Hz,  $CH_2-N$  hexylspacer), 3.55 - 3.68 (m, 4H,  $CH_2-O$  hexylspacer, 2 x CH isopropylamino), 3.71 - 3.82 (m, 2H,  $CH_2$  cyanoethyl), 5.00 (s, 2H,  $CH_2$  F-Bn), 5.74 (t, 1H,  $J = 5.5$  Hz, NH), 7.20 (d, 2H,  $J = 7.9$  Hz,  $H_{arom}$ ), 7.27 (d, 2H,  $J = 7.9$  Hz,  $H_{arom}$ );  $^{13}C$  NMR (100 MHz,  $CD_3CN$ ):  $\delta = 21.1$  (d,  $J = 7$  Hz,  $CH_2$  cyanoethyl), 25.0 (d,  $J = 7$  Hz, 4 x  $CH_3$  isopropylamino), 26.5 ( $CH_2$  hexylspacer), 26.7 ( $F_{17}C_8CH_2CH_2-$ ), 27.2 ( $CH_2$  hexylspacer), 30.7 ( $CH_2$  hexylspacer), 31.9 (d,  $J = 7$  Hz,  $CH_2$  hexylspacer), 33.1 (t,  $J = 22$  Hz,  $F_{17}C_8CH_2CH_2-$ ), 41.6 ( $CH_2-N$  hexylspacer), 43.8 (d,  $J = 12$  Hz, 2 x CH isopropylamino), 59.3 (d,  $J = 19$  Hz,  $CH_2$  cyanoethyl), 64.3 (d,  $J = 17$  Hz,  $CH_2-O$  hexylspacer), 66.4 ( $CH_2$  F-Bn), 119.5 ( $C_q$  cyanoethyl), 129.1, 129.4 ( $CH_{arom}$ ), 136.9 ( $C_q$  F-Bn), 139.9 ( $C_q$  F-Bn), 157.4 (C=O carbamate).



#### Fluororous hexa(2-benzyl)glycerol phosphate (**20**)

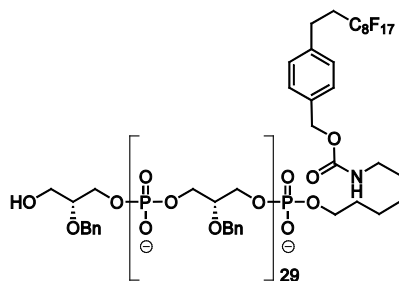
Automated solid phase TA synthesis was performed as described above using 15  $\mu$ mol of immobilized glycerol (**19**). After five consecutive elongation cycles using phosphoramidite **18a** a capping step with fluororous phosphoramidite **17** was performed. After ammonia treatment the crude product was purified with F-SPE affording hexamer **20** (17.2 mg, 7.6  $\mu$ mol, 51%) as an amorphous white solid.  $^{31}P$  NMR (161.7 MHz, 3/2  $D_2O/CD_3OD$ ):  $\delta = -0.2$  (4P), 0.0 (2P);  $^1H$  NMR (600 MHz, 3/2  $D_2O/CD_3OD$ ,  $T = 328K$ ):  $\delta = 1.16$  - 1.29 (m, 4H, 2 x  $CH_2$  hexylspacer), 1.34 - 1.39 (m, 2H,  $CH_2$  hexylspacer), 1.45 - 1.51 (m, 2H,  $CH_2$  hexylspacer), 2.29 - 2.39 (m, 2H,  $F_{17}C_8CH_2CH_2-$ ), 2.82 (t, 2H,  $J = 7.9$  Hz,  $F_{17}C_8CH_2CH_2-$ ), 2.99 - 3.05 (m, 2H,  $CH_2-N$  hexylspacer), 3.57 - 3.68 (m, 3H, CH glycerol,  $CH_2$  glycerol), 3.72 - 3.99 (m, 29H, 5 x CH glycerol, 11 x  $CH_2$  glycerol,  $CH_2-O$  hexylspacer), 4.51 - 4.63 (m, 12H, 6 x  $CH_2$  Bn), 5.01 (s, 2H,  $CH_2$  F-Bn), 7.11 - 7.33 (m, 34H,  $H_{arom}$ ); HRMS:  $C_{84}H_{102}F_{17}NO_{33}P_6 + NH_4^+$  requires 2179.4827, found 2179.4840.



### Fluorous (2-benzyl)glycerol phosphate 20-mer (21)

Automated solid phase TA synthesis was performed as described above using 15  $\mu\text{mol}$  of immobilized glycerol (**19**). After nineteen consecutive elongation cycles using phosphoramidite **18a** a capping step with fluorous phosphoramidite **17** was performed. After ammonia treatment the crude product was purified with F-SPE. In addition, the purified oligomer was eluted through a small column containing Dowex

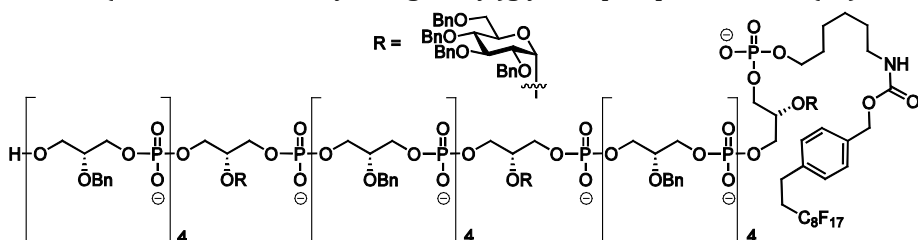
$\text{Na}^+$  cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in  $\text{H}_2\text{O}$ , flushed with  $\text{H}_2\text{O}$  and MeOH before use). Lyophilization afforded 20-mer **21** (22.2 mg, 3.69  $\mu\text{mol}$ , 25%) as an amorphous white solid.  $^{31}\text{P}$  NMR (161.7 MHz, 3/2  $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ ):  $\delta = 1.2$  (18P), 1.3 (2P);  $^1\text{H}$  NMR (400 MHz, 3/2  $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ ,  $T = 328\text{K}$ ):  $\delta = 1.10 - 1.34$  (m, 6H, 3 x  $\text{CH}_2$  hexylspacer), 1.39 - 1.49 (m, 2H,  $\text{CH}_2$  hexylspacer), 2.11 - 2.26 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2-$ ), 2.65 - 2.71 (m 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2-$ ), 2.92 - 2.98 (m, 2H,  $\text{CH}_2\text{-N}$  hexylspacer), 3.53 - 3.99 (m, 102H, 20 x CH glycerol, 40 x  $\text{CH}_2$  glycerol,  $\text{CH}_2\text{-O}$  hexylspacer), 4.47 - 4.59 (m, 40H, 20 x  $\text{CH}_2$  Bn), 4.94 (bs, 2H,  $\text{CH}_2$  F-Bn), 7.00 - 7.30 (m, 104H,  $\text{H}_{\text{arom}}$ ); HRMS:  $[\text{C}_{224}\text{H}_{284}\text{F}_{17}\text{NO}_{103}\text{P}_{20} + 2\text{NH}_4]^{2+}$  requires 2808.1120, found 2808.1094.



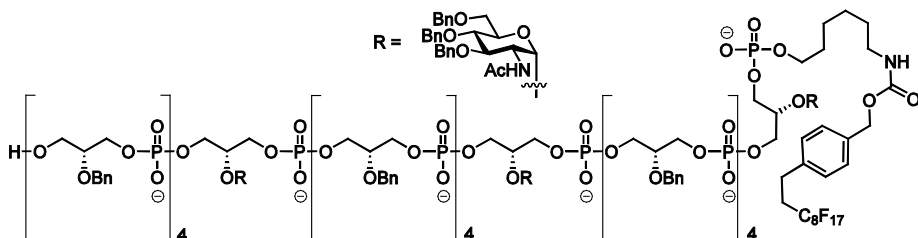
### Fluorous (2-benzyl)glycerol phosphate 30-mer (22)

Automated solid phase TA synthesis was performed as described above using 15  $\mu\text{mol}$  of immobilized glycerol (**19**). After 29 consecutive elongation cycles using phosphoramidite **18a** a capping step with fluorous phosphoramidite **17** was performed. After ammonia treatment the crude product was purified with RP-HPLC (C18). In addition, the purified oligomer was eluted through a small column containing Dowex  $\text{Na}^+$  cation-exchange resin (type:

50WX4-200, stored on 0.5 M NaOH in  $\text{H}_2\text{O}$ , flushed with  $\text{H}_2\text{O}$  and MeOH before use). Lyophilization afforded 30-mer **22** (14.6 mg, 1.68  $\mu\text{mol}$ , 11%) as an amorphous white solid.  $^{31}\text{P}$  NMR (161.7 MHz, 7/3  $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ ):  $\delta = 1.0$  (28P), 1.1 (2P);  $^1\text{H}$  NMR (400 MHz, 7/3  $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ ,  $T = 328\text{K}$ ):  $\delta = 1.01 - 1.29$  (m, 6H, 3 x  $\text{CH}_2$  hexylspacer), 1.34 - 1.41 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.85 - 2.01 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2-$ ), 2.43 - 2.51 (m 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2-$ ), 2.86 - 2.95 (m, 2H,  $\text{CH}_2\text{-N}$  hexylspacer), 3.52 - 4.03 (m, 152H, 30 x CH glycerol, 60 x  $\text{CH}_2$  glycerol,  $\text{CH}_2\text{-O}$  hexylspacer), 4.47 - 4.59 (m, 60H, 30 x  $\text{CH}_2$  Bn), 4.90 (bs, 2H,  $\text{CH}_2$  F-Bn), 6.92 - 7.29 (m, 154H,  $\text{H}_{\text{arom}}$ ); HRMS:  $[\text{C}_{324}\text{H}_{414}\text{F}_{17}\text{NO}_{153}\text{P}_{30} + 3\text{NH}_4]^{3+}$  requires 2691.9206, found 2691.9191.

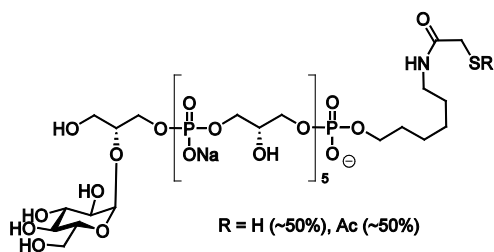
**Fluorous tri(2,3,4,6-tetra-*O*-benzyl- $\alpha$ -D-glucosyl)glycerol phosphate 15-mer (23)**

Automated solid phase TA synthesis was performed as described above using 9  $\mu\text{mol}$  of immobilized glycerol (**19**). After 3 consecutive elongation cycles using phosphoramidite **18a** one elongation cycle was performed using glucosylglycerol phosphoramidite **18b**. This was followed by 4 elongations using glycerol amidite **18a** and one using glucosylglycerol amidite **18b**. The latter five-step elongation cycle was repeated once, which was followed by capping with fluorous phosphoramidite **17**. After ammonia treatment the crude product was purified with RP-HPLC (C4). Lyophilization afforded 15-mer **23** (13.5 mg, 1.88  $\mu\text{mol}$ , 21%,  $\text{Et}_3\text{NH}^+$ -form) as an amorphous white solid.  $^{31}\text{P}$  NMR (161.7 MHz, 1/1  $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ ,  $T = 328\text{K}$ ):  $\delta = 1.8 - 2.4$  (15P);  $^1\text{H}$  NMR (400 MHz, 1/1  $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ ,  $T = 328\text{K}$ ):  $\delta = 1.05 - 1.31$  (m, 141H, 3 x  $\text{CH}_2$  hexylspacer, 45 x  $\text{CH}_3$   $\text{Et}_3\text{NH}^+$ ), 1.40 - 1.47 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.83 - 1.95 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2$ -), 2.38 - 2.47 (m 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2$ -), 2.91 - 3.00 (m, 2H,  $\text{CH}_2$ -N hexylspacer), 3.09 (q, 90H,  $J = 7.3$  Hz, 45 x  $\text{CH}_2$   $\text{Et}_3\text{NH}^+$ ), 3.34 - 4.25 (m, 95H, 15 x CH glycerol, 30 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$ -O hexylspacer, 3 x H-2, 3 x H-3, 3 x H-4, 3 x H-5, 3 x H-6, 3 x H-6'), 4.51 - 4.67 (m, 48H, 24 x  $\text{CH}_2$  Bn), 4.81 (bs, 2H,  $\text{CH}_2$  F-Bn), 5.09 - 5.17 (m, 3H, 3 x H-1), 6.79 - 7.26 (m, 124H,  $\text{H}_{\text{arom}}$ ); LC-MS:  $[\text{C}_{255}\text{H}_{303}\text{F}_{17}\text{NO}_{93}\text{P}_{15} + 2\text{H}]^{2+}$  requires 2830.8, found 2830.6.

**Fluorous tri(2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy- $\alpha$ -D-glucosyl)glycerol phosphate 15-mer (24)**

Automated solid phase TA synthesis was performed as described above using 9  $\mu\text{mol}$  of immobilized glycerol (**19**). After 3 consecutive elongation cycles using phosphoramidite **18a** one elongation cycle was performed using *N*-acetylglucosaminylglycerol phosphoramidite **18c**. This was followed by 4 elongations using glycerol amidite **18a** and one using *N*-acetylglucosaminylglycerol amidite **18c**. The latter five-step elongation cycle was repeated once, which was followed by capping with fluorous phosphoramidite **17**. After ammonia treatment the crude product was purified with RP-HPLC (C4). Lyophilization afforded 15-mer **24** (15.0 mg, 2.13  $\mu\text{mol}$ , 24%,  $\text{Et}_3\text{NH}^+$ -form) as an amorphous white solid.  $^{31}\text{P}$  NMR (161.7 MHz, 1/1  $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ ,  $T = 328\text{K}$ ):  $\delta = 0.3 - 0.7$  (13P), 0.8 (1P), 1.0 (1P);  $^1\text{H}$  NMR (400 MHz, 1/1  $\text{D}_2\text{O}/\text{CD}_3\text{OD}$ ,  $T = 328\text{K}$ ):  $\delta = 1.17 - 1.41$  (m, 141H, 3 x  $\text{CH}_2$  hexylspacer, 45 x  $\text{CH}_3$   $\text{Et}_3\text{NH}^+$ ), 1.48 - 1.56 (m, 2H,  $\text{CH}_2$  hexylspacer), 1.92 - 2.01 (3 x s, 9H, 3 x  $\text{NHAc}$ ), 2.11 - 2.26 (m, 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2$ -), 2.67 - 2.73 (m 2H,  $\text{F}_{17}\text{C}_8\text{CH}_2\text{CH}_2$ -), 2.94 - 3.02 (m, 2H,  $\text{CH}_2$ -N hexylspacer), 3.09 (q, 90H,  $J = 7.3$  Hz, 45 x  $\text{CH}_2$   $\text{Et}_3\text{NH}^+$ ), 3.50 - 4.13 (m, 95H, 15 x CH glycerol, 30 x  $\text{CH}_2$  glycerol,  $\text{CH}_2$ -O hexylspacer, 3 x H-2, 3 x H-3, 3 x H-4, 3 x H-5, 3 x H-6, 3 x H-6'), 4.20 - 4.58 (m, 42H, 21 x  $\text{CH}_2$

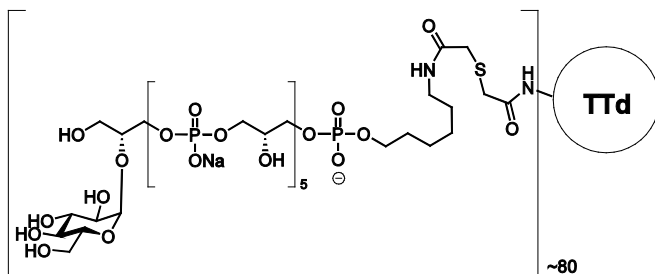
Bn), 4.92 (bs, 2H, CH<sub>2</sub> F-Bn), 4.94 - 4.97 (m, 3H, 3 x H-1), 6.92 - 7.30 (m, 109H, H<sub>arom</sub>); LC-MS: [C<sub>240</sub>H<sub>294</sub>F<sub>17</sub>N<sub>4</sub>O<sub>93</sub>P<sub>15</sub> + 3H]<sup>3+</sup> requires 1838.5, found 1838.4.



### (2-thioacetyl)acetyl glucosyl hexaglycerol phosphate (28)

To a solution of **25** (8.7 mg, 6.6  $\mu$ mol) in 0.5 ml freshly prepared tetraborate buffer (0.1 M, pH 8.5) was added SATA (**27**, 30 mg, 0.13 mmol, dissolved in 300  $\mu$ l DMF). The mixture was allowed to stir overnight after which it was filtered and the target compound isolated by size exclusion chromatography (HW40, 50 mM Et<sub>3</sub>NHOAc). The purified

product was lyophilized twice before it was dissolved in H<sub>2</sub>O (~0.5 ml) and eluted to a small column containing Dowex Na<sup>+</sup> cation-exchange resin (type: 50WX4-200, stored on 0.5 M NaOH in H<sub>2</sub>O, flushed with H<sub>2</sub>O and MeOH before use). Lyophilization afforded the target oligomer (**28**, 9.2 mg, ~6.4 mmol, 96%) as a 1:1 mixture of acetate and free thiol. <sup>31</sup>P NMR (161.7 MHz, D<sub>2</sub>O):  $\delta$  = 1.2 - 1.3 (5P), 1.3 - 1.4 (1P); <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O):  $\delta$  = 1.23 - 1.34 (m, 4H, 2 x CH<sub>2</sub> hexylspacer), 1.40 - 1.51 (m, 2H, CH<sub>2</sub> hexylspacer), 1.52 - 1.60 (m, 2H, CH<sub>2</sub> hexylspacer), 2.33 (s, ~1.5H, CH<sub>3</sub> SAc, ~50% on), 3.08 - 3.19 (m, 2H, CH<sub>2</sub>-N hexylspacer), 3.34 (t, 1H, *J* = 9.4 Hz, H-4), 3.37 (s, ~1H, CH<sub>2</sub>SH), 3.46 (dd, 1H, *J* = 3.6 Hz, 9.8 Hz, H-2), 3.56 (s, ~1H, CH<sub>2</sub>SAc), 3.64 - 4.01 (m, 36H, 6 x CH glycerol, 12 x CH<sub>2</sub> glycerol, CH<sub>2</sub>-O hexylspacer, H-3, H-5, H-6, H-6'), 5.10 (d, 1H, *J* = 3.5 Hz, H-1).



### TA-TTd conjugate (30)

To a solution of modified TA **28** (9.0 mg, 6.2  $\mu$ mol) in H<sub>2</sub>O (250  $\mu$ l) were added bromoacetylated TTd (**29**, 7.0 mg, 47 nmol, in 2.0 ml PBS buffer pH 6.0, containing 5 mM EDTA, freshly prepared as described by van der Ley *et*

*al.*)<sup>ref hoogerhout</sup> and hydroxylamine hydrochloride (2.0 M in PBS, 25  $\mu$ l, 50  $\mu$ mol). The mixture was mixed gently and allowed to stand overnight at room temperature after which 2-aminoethanethiol hydrochloride (18 mM in PBS pH 6.0, 225  $\mu$ l, 4.0  $\mu$ mol) was added. After stirring overnight, the crude modified TTd (**30**, in a total volume of 2.5 ml) was equally divided over two PD-10 columns, which were pre-equilibrated with PBS buffer (pH 7.2). The columns were eluted with PBS (pH 7.2, 3.5 ml) and the fractions containing the modified protein combined. The purified conjugate **30** was analyzed on sugar content by a phenol/sulfuric acid assay<sup>ref</sup> (using TA **25** as external standard, protein/sugar ratio: ~1.2/1) and stored in solution (PBS, 7 ml, ~1 mg protein/ml) at 4 °C.<sup>ref hoogerhout</sup>

## References

1. Fischer, W. *Adv. Microb Physiol.* **1988**, 29, 233-302.
2. Naumova, I.B.; Shashkov, A.S.; Tul'skaya, E.M.; Streshinskaya, G.M.; Kozlova, Y.I.; Potekhina, N.V.; Evtushenko, L.I.; Stackebrandt, E. *FEMS Microbiol. Rev.* **2001**, 25, 269-283.
3. Neuhaus, F.C.; Baddiley, J. *Microbiol. Mol. Biol. Rev.* **2003**, 67, 686-723.
4. Weidenmaier, C.; Peschel, A. *Nat. Rev. Microbiol.* **2008**, 6, 276-287.
5. Swoboda, J.G.; Campbell, J.; Meredith, T.C.; Walker, S. *ChemBioChem* **2010**, 11, 35-45.
6. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Sava, I.; Ye, L.; Bychowska, A.; Holst, O.; Huebner, J. *Plos One* **2011**, 6, e17839.
7. Theilacker, C.; Krueger, W.A.; Kropec, A.; Huebner, J. *Vaccine*, **2004**, 22S, 31-38.
8. Sava, I.G.; Heikens, E.; Huebner, J. *Clin. Microbiol. Inf.* **2010**, 16, 533-540.
9. Theilacker, C.; Kaczynski, Z.; Kropec, A.; Fabretti, F.; Sange, T.; Holst, O.; Huebner, J. *Infect. Immun.* **2006**, 74, 5703-5712.
10. Theilacker, C.; Kropec, A.; Hammer, F.; Sava, I.; Wobser, D.; Sakinc, T.; Codée, J.D.C.; Hogendorf, W.F.J.; van der Marel, G.A.; Huebner, J. *J. Infect. Dis.* **2012**, 205, 1076-1085.
11. Hogendorf, W.F.J.; Van den Bos, L.J.; Overkleeft, H.S.; Codée, J.D.C.; van der Marel, G.A. *Bioorg. Med. Chem.* **2010**, 18, 3668-3678.
12. Hogendorf, W.F.J.; Meeuwenoord, N.; Overkleeft, H.S.; Filippov, D.V.; Laverde, D.; Kropec, A.; Huebner, J.; van der Marel, G.A.; Codée, J.D.C. *Chem. Commun.* **2011**, 47, 8961-8963.
13. Schmidt, R.R.; Pedersen, C.M.; Qiao, Y.; Zähringer, U. *Org. Biomol. Chem.* **2011**, 9, 2040-2052.
14. Figueroa-Perez, I.; Stadelmaier, A.; Morath, S.; Hartung, T.; Schmidt, R.R. *Tetrahedron: Asymm.* **2005**, 16, 493-506.
15. Hogendorf, W.F.J.; Lameijer, L.N.; Beenakker, T.J.M.; Overkleeft, H.S.; Filippov, D.V.; Codée, J.D.C.; Van der Marel, G.A. *Org. Lett.* **2012**, 14, 848-851.
16. Hogendorf, W.F.J.; Kropec, A.; Filippov, D.V.; Overkleeft, H.S.; Huebner, J.; Van der Marel, G.A. *Carbohydr. Res.* **2012**, 356, 142-151.
17. Tripathy, S.; Misra, K.; Sanghvi, Y.S. *Org. Prep. Proc.* **2005**, 37, 257-263.
18. Pearson, W.H.; Berry, D.A.; Stoy, P.; Jung, K.-Y.; Sercel, A.D. *J. Org. Chem.* **2005**, 70, 7114-7122.
19. Beller, C.; Bannwarth, W. *Helv. Chim. Acta* **2005**, 88, 171-179.
20. Filippov, D.V.; van Zoelen, D.J.; Oldfield, S.P.; van der Marel, G.A.; Overkleeft, H.S.; Drijfhout, J.W.; Van Boom, J.H. *Tetrahedron Lett.* **2002**, 43, 7809-7812.
21. Verez-Bencomo, V.; Fernández-Santana, V.; Hardy, E.; Toledo, M.E.; Rodríguez, M.C.; Heynngnezz, L.; Rodriguez, A.; Baly, A.; Herrera, L.; Izquierdo, M.; Villar, A.; Valdés, Y.; Cosme, K.; Deler, M.L.; Montane, M.; Garcia, E.; Ramos, A.; Aguilar, A.; Medina, E.; Toraño, G.; Sosa, I.; Hernandez, I.; Martínez, R.; Muzachio, A.; Carmenates, A.; Costa, L.; Cardoso, F.; Campa, C.; Diaz, M.; Roy, R. *Science* **2004**, 305, 522-525.
22. Manuscript in preparation.
23. Drijfhout, J.W.; Bloemhoff, W.; Poolman, J.T.; Hoogerhout, P. *Anal. Biochem.* **1990**, 187, 349-354.
24. Van der Ley, P.; Heckels, J.E.; Virji, M.; Hoogerhout, P.; Poolman, J.T. *Infect. Immun.* **1991**, 59, 2963-2971.
25. Fournier, E. *Current Protocols in Food Analytical Chemistry*, John Wiley & Sons Inc.: New York, USA, **2001**.

# Nederlandse Samenvatting

## Synthetische methodologiën naar glycerol teichoïnezuren

Teichoïnezuren kunnen in het algemeen worden gekarakteriseerd als polymeren opgebouwd uit repeterende gesubstitueerde glycerol of ribitol eenheden, die door middel van fosfodiësters verbonden zijn. Het substitutiepatroon kan per repeterende eenheid verschillen en kan bestaan uit verschillende koolhydraatstructuren en/of D-alanine esters. Teichoïnezuren komen voor in de celwand van Gram-positieve bacteriën en zijn kenmerkend voor een bacteriesoort. Teichoïnezuren vervullen een scala van functies die voor een bacterie van levensbelang zijn en spelen een rol bij de interactie van de bacterie met de omgeving. Zo kunnen teichoïnezuren worden herkend door het menselijk immuunsysteem.

Door de toenemende resistentie van bacteriën tegen antibiotica wordt er veel aandacht besteed aan de ontwikkeling van andere geneeswijzen zoals nieuwe soorten van (synthetische) vaccins. Teichoïnezuren kunnen vanwege hun antigene eigenschappen wellicht een rol spelen bij de ontwikkeling van dergelijke vaccins. Op grond van de mogelijke antigene eigenschappen van teichoïnezuren is een onderzoek gestart om deze verbindingen synthetisch toegankelijk te maken en om de immunologische eigenschappen van deze gedefinieerde, synthetische teichoïnezuren vast te stellen.

Dit proefschrift beschrijft de ontwikkeling van drie methoden voor de synthese van glycerol teichoïnezuur fragmenten van verschillende lengte en met verschillende glycosylerings patronen. In samenwerking met de groep van Professor Huebner (Freiburg Universitair Medisch Centrum) werden de gesynthetiseerde teichoïnezuur fragmenten geëvalueerd op hun immunologische eigenschappen.

In het inleidende **hoofdstuk 1** wordt aandacht besteed aan de ontdekking, de structurele classificatie en de fysiologische en immunologische aspecten van teichoïnezuren. Tevens wordt een overzicht gegeven van bestaande syntheseswegen naar fragmenten van teichoïnezuren van verschillende bacteriële oorsprong.

De synthese van een 2'- $\alpha$ -kajibiosyl (een  $\alpha$ -1 $\rightarrow$ 2 verknoopt glucosyl disaccharide) bevattend fragment van het teichoïnezuur van *Enterococcus faecalis* is het onderwerp van **hoofdstuk 2**. In oplossing werd met behulp van fosforamidiet chemie een glycerol fosfaat hexameer met één 2'- $\alpha$ -kajibiosyl eenheid gesynthetiseerd. Het eerste stadium van de synthese route omvat de bereiding van de orthogonaal beschermde glycerol fosforamidieten, zoals die voorzien van de 2'- $\alpha$ -kajibiosyl eenheid. Vanuit dibenzylglycerol wordt het hexameer vervolgens stap voor stap opgebouwd doormiddel van de volgende verlengingsprocedure: 1) vorming van een fosfiettriestier door koppeling van een fosforamidiet met het vrije alcohol. 2) Oxidatie van het fosfiet triester intermediair. 3) Het ontschermen van de 4,4'-dimethoxytrityl groep, met als resultaat het verkrijgen van een nieuw vrij alcohol. Deze 3-staps verlengings-



procedure vormt de basis van alle oligoglycerol fosfaat syntheses beschreven in dit proefschrift.

De fosforamidiëchemie beschreven in **hoofdstuk 2** kon worden toegepast in onderzoek naar een geautomatiseerde vaste drager synthese van teichoïnezuur fragmenten. In **hoofdstuk 3** wordt beschreven hoe een aangepaste DNA synthesizer kan worden gebruikt om enkele milligrammen van de teichoïnezuur fragmenten efficiënt en snel te produceren. Met deze techniek werd een kleine bibliotheek aan verbindingen verkregen bestaande uit 6-, 10-, 14- en 20-meren glycerol-1,3-fosfaat en twee geglycosyleerde hexaglycerol fosfaat teichoïnezuur fragmenten. De verkregen fragmenten werden getest op hun affiniteit voor antilichamen in immuunserum opgewekt in konijnen tegen geïsoleerd lipoteichoïnezuur van *E. faecalis*. Hieruit bleek dat het 20-meer glycerol fosfaat en de twee geglycosyleerde fragmenten het meest effectief aan de antilichamen binden.

**Hoofdstuk 4** doet verslag van een nieuwe syntheseprocedure waarbij teichoïnezuur fragmenten worden gesynthetiseerd en geïsoleerd met behulp van een *fluorous* (perfluoroalkyl) beschermende groep. De perfluorooctylpropylsulfonylethyl (F-Pse) groep werd onderzocht als fosfaat beschermende groep. Het hoge gehalte aan fluoratomen in deze groep zorgt er voor dat een beschermd teichoïnezuur oligomeer voorzien van de F-Pse groep uitstekend bindt aan ge(per)fluoreerde materialen zoals *fluorous* silica, zodat zuivering na iedere elongatie sequentie kan plaatsvinden op basis van deze eigenschap. Met deze techniek werden enkele tientallen milligrammen van een 12-meer glycerol fosfaat, twee hexaglycerolfosfaten met (*N*-acetyl)glucosaminy substituties en een hexameer van (*N*-acetyl)glucosaminyglycerol fosfaat verkregen. Een belangrijk verschil met de fragmenten beschreven in **hoofdstuk 2 en 3** is dat de teichoïnezuren beschreven in **hoofdstuk 4** een terminale fosfaat bevatten. De fragmenten bleken in de immunologische evaluatie niet actief te zijn.

Om door middel van zuivering met behulp van *fluorous* silica, teichoïnezuur fragmenten zonder terminale fosfaat toegankelijk te maken werd een alternatieve hydroxyl *fluorous* beschermende groep ontwikkeld. **Hoofdstuk 5** behandelt de synthese van enkele geglycosyleerde teichoïnezuur fragmenten met behulp van een *fluorous* beschermende groep welke is gebaseerd op een succinyl ester. Tevens wordt uitgelegd hoe een “zuur-stabiel” 2'-(tetra-*O*-benzyl- $\alpha$ -glucosyl)glycerol fosforamidië kan worden verkregen. Deze glucosyl substitutie is stabiel tijdens de standaard detrityleringscondities en kan daardoor op een iedere positie in een teichoïnezuur fragment worden ingebouwd.

In **hoofdstuk 6** wordt beschreven hoe het immunologisch meest interessante teichoïnezuur fragment (geglycosyleerd hexaglycerol fosfaat) wordt geconjugeerd aan BSA, een immunogeen drager-eiwit. Met dit experimentele vaccin werden konijnen geïmmuniseerd en met behulp van opsonofagocytische testen werd aangetoond dat het serum verkregen van deze konijnen antilichamen bevat welke efficiënt aan teichoïnezuren van *E. faecalis* bindt. Het bleek dat het konijnen antiserum zelfs in zeer

verdunde toestand in staat was *E. faecalis* te opsonizeren en dus potentieel als therapeutisch vaccin zou kunnen functioneren. Verrassend genoeg werden door dit serum ook *E. faecium* (in meerdere mate) en *Staphylococcus aureus* (in mindere mate) geopsoniseerd. Uit een endocarditis model experiment in ratten bleek dat het eerder genoemde antiserum gebruikt kan worden om passief te immunizeren en zo bescherming te bieden tegen infecties veroorzaakt door *E. faecalis*.



# List of Publications

## **Light fluororous synthesis of glucosylated glycerol teichoic acids**

Hogendorf, W.F.J.; Kropec, A.; Filippov, D.V.; Overkleeft, H.S.; Huebner, J.; van der Marel, G.A.; Codée, J.D.C.

*Carbohydr. Res.* **2012**, *356*, 142-151.

## **Fluororous linker facilitated synthesis of teichoic acid fragments**

Hogendorf, W.F.J.; Lameijer, L.N.; Beenakker, T.J.M.; Overkleeft, H.S.; Filippov, D.V.; Codée, J.D.C.; Van der Marel, G.A.

*Org. Lett.* **2012**, *14*, 848-851.

## **Protection against *S. aureus* by antibody to the polyglycerolphosphate backbone of heterologous lipoteichoic acid**

Theilacker, C.; Kropec, A.; Hammer, F.; Sava, I.; Wobser, D.; Sakinc, T.; Codée, J.D.C.; Hogendorf, W.F.J.; Van der Marel, G.A.; Huebner, J.

*J. Infect. Dis.* **2012**, *205*, 1076-1085.

## **Synthetic LTA mimetics and use thereof as vaccine component for therapy and/or prophylaxis against Gram-positive infections**

Theilacker, C.; Huebner, J.; Hogendorf, W.F.J.; Van der Marel, G.A.; Codée, J.D.C.

**2012**, *Patent pending*.

## **Automated solid phase synthesis of teichoic acids**

Hogendorf, W.F.J.; Meeuwenoord, N.; Overkleeft, H.S.; Filippov, D.V.; Laverde, D.; Kropec, A.; Huebner, J.; Van der Marel, G.A.; Codée, J.D.C.

*Chem. Commun.* **2011**, *47*, 8961-8963.

## **C-Glycosylation starting from unprotected O-glycosides**

La Ferla, B.; Cipolla, L.F.; Hogendorf, W.F.J.; Nicotra, F.

*Carbohydrate Chemistry: Proven Methods*, **2011**, vol. 1, Chapter 8, 83–89.

## **synthesis of an $\alpha$ -kojibiosyl substituted glycerol teichoic acid hexamer**

Hogendorf, W.F.J.; Van den Bos, L.J.; Overkleeft, H.S.; Codée, J.D.C.; Van der Marel, G.A.

*Bioorg. Med. Chem.* **2010**, *18*, 3668-3678.

**The synthesis of a menthol derivative of 2-aminopurine as a fluorescent DNA lesion**

Hogendorf, W.F.J.; Verhagen, C.P.; Malta, E.; Goosen, N.; Overkleeft, H.S.; Filippov, D.V.; Van der Marel, G.A.

*Tetrahedron* **2009**, *65*, 10430-10435.

**Stereoselective synthesis of L-guluronic acid alginates**

Dinkelaar, J.; Van den Bos, L.J.; Hogendorf, W.F.J.; Lodder, G.; Overkleeft, H.S.; Codée, J.D.C.; Van der Marel, G.A.

*Chem. Eur. J.* **2008**, *14*, 9400-9411.

Cover Page



Universiteit Leiden



The handle <http://hdl.handle.net/1887/20172> holds various files of this Leiden University dissertation.

**Author:** Hogendorf, Wouter Frederik Johan

**Title:** Synthetic methods to glycerol teichoic acids

**Issue Date:** 2012-11-22

# Curriculum Vitae

Wouter Frederik Johan Hogendorf werd op 30 augustus 1983 geboren te Leiden. Na het behalen van het HAVO-diploma aan het Aquino College te Leiden in 2000 werd in dat jaar begonnen met de opleiding Organische Chemie aan de Hogeschool Leiden. Van september 2004 tot en met mei 2005 werd in het kader van een afstudeerstage onderzoek verricht aan de Technische Universiteit Delft bij de vakgroep Biokatalyse en Organische Chemie onder leiding van dr. Fred van Rantwijk en prof. dr. Roger A. Sheldon. Dit onderzoek omvatte de evaluatie van diverse nitrilases voor de (partiële) hydrolyse van een reeks nitril bevattende verbindingen. Na het succesvol afronden van de opleiding Organische Chemie aan de Hogeschool Leiden in augustus 2005 werd aangevangen met de master opleiding 'Chemistry' van de Universiteit Leiden met als specialisatie 'Design and Synthesis'. De hoofdvakstage werd uitgevoerd van oktober 2005 tot en met augustus 2006 onder begeleiding van dr. Jasper Dinkelaar bij de vakgroep Bio-organische Synthese van prof. dr. Gijs A. van der Marel en prof. dr. Herman S. Overkleef. Dit onderzoeksproject betrof de evaluatie van L-gulose en L-guluronzuur derivaten in de synthese van alginaat fragmenten, potentiële Toll-like receptor liganden. De bijvakstage werd verricht van februari 2007 tot en met juni 2007 onder begeleiding van Carlo P. Verhagen en dr. Dmitri V. Filippov. Dit onderzoek betrof de synthese van een gemodificeerd fluorescent DNA fragment in het kader van het ophelderen van 'nucleotide excision repair', een DNA reparatie mechanisme. Het master diploma werd in januari 2008 behaald.

In januari 2008 werd hij benoemd als assistent in opleiding aan de Universiteit Leiden en werd begonnen met het in dit proefschrift beschreven onderzoek, dat werd uitgevoerd in de vakgroep Bio-organische Synthese onder begeleiding van dr. Jeroen D.C. Codée, dr. Dmitri Filippov, prof. dr. Gijs A. van der Marel en prof. dr. Herman S. Overkleef. Gedeelten van dit onderzoek werden mondeling gepresenteerd op het '16<sup>th</sup> European Carbohydrate Symposium' (juni 2011 te Sorrento, Italië) en tweemaal op het 'Wageningen Symposium on Organic Chemistry' (april 2010 en april 2012 respectievelijk, te Wageningen). Delen van dit onderzoek zijn op poster gepresenteerd op de jaarlijkse NWO-CW 'Design and Synthesis' symposia in Lunteren (oktober 2009 en oktober 2010). Tevens nam de auteur van dit proefschrift in juli 2009 deel aan de HRSMC summer school 'New Horizons in Synthetic Methodologie' te Maastricht. Vanaf oktober 2012 is hij werkzaam als post-doctoraal onderzoeker in de onderzoeksgroep onder leiding van Professor Bols aan de universiteit van Kopenhagen in Denemarken.